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TEXTBOOK OF BIOLOGICAL CHEMISTRY

BY

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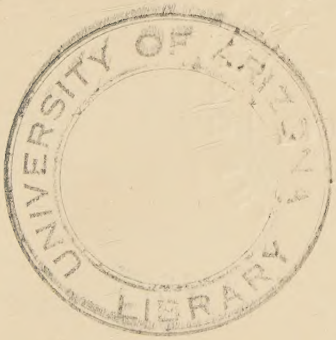

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PREFACE

In preparing this book it has been my aim to set before the students as briefly and simply as possible the principal facts and theories of biological chemistry. In striving to attain this objective it has manifestly been impossible to include a great amount of material that is of interest and importance to the biochemist. The book is meant first and last for the elementary student and not for reference. I make no pretension of having written an exhaustive account of the subjects which are presented, and I believe that for a book of this sort the ability to omit is fully as important as the ability to include.

I wish to acknowledge my indebtedness to Dr. Aaron Bodansky, whose criticisms have been of great value, to Cleveland Abbe, Jr., Ph.D., and to my wife for the correction of chemist's English.

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INTRODUCTION

Definition of Biological Chemistry.—Biological, or physiological chemistry, is the chemistry of living things, or their products. It includes the inorganic, organic, analytical, electro- and physical chemistry of plants and animals. In biological chemistry, chemical combination and chemical reaction, the basis of all chemistry, depend upon the ability of some atoms to lose electrons and of other atoms to take on electrons.

Aims and Ideals of Biological Chemistry.—The aim of the biological chemist is to explain the chemical composition of plants and animals and the chemical structure of the compounds present. In collaboration with the physiologist he must also explain such phenomena as the digestion and absorption of foods, respiration, the oxidation or catabolism of food materials, muscular contraction, glandular secretion, growth, regeneration, reproduction and the conduction of nerve impulses. While a vast amount of progress has already been made in the study of these problems by scientists of all nationalities, it must be admitted that only the surface has been laid bare. Much remains to be done. We are today unable to explain fully a single one of the processes listed above, indeed concerning the majority of these processes our present explanations are admitted to be entirely inadequate.

The domain included under the term biological chemistry is an extensive one. Thus, the organic chemist who seeks to explain the chemical structure of proteins, phosphatides, bile pigments, or polysaccharides, can be said to be working in biological chemistry. The same thing can also be said of the analytical chemist who perfects new or more rapid

methods for the analysis of biological substances, or publishes analytical data dealing with the organic or inorganic constituents of blood, urine or tissue; of the physical chemist who explains vital phenomena in terms of osmotic pressure, surface tension, hydrogen ion concentration, or viscosity; of the colloid chemist who studies the colloidal properties of plant and animal tissues and fluids, the swelling of gels and the precipitation of proteins; of the dietitian who investigates the biological food values of individual proteins, or the isolation of vitamins; or of the worker in the field of animal calorimetry who measures the production of energy and studies the influence of various factors upon the metabolic rate.

Closely related to physiological chemistry is the science of pathological chemistry, which concerns itself with the effect of disease upon the chemistry of the organism. The recent discovery of insulin and its use in diabetes mellitus is a noteworthy example of research in this and in closely allied fields. The isolation of adrenalin and thyroxin and the preparation of active extracts of the pituitary, of the ovaries and of the parathyroid glands, followed by investigation of the influence of these substances upon normal and diseased animals, are instances of what is being accomplished in the field of endocrinology, a subject of interest to physiologist and biochemist alike.

The Importance of Biological Chemistry.—The courses in biological chemistry offered in the curricula of our colleges have been devised largely for the student in medicine, but there are other fields, such as clinical analysis, dietetics, bacteriology, animal husbandry and dairy industry, wherein a substantial knowledge of the fundamental principles of biological chemistry can be said to be indispensible. Whereas the medical student of a generation ago was required to possess no more than a minimal knowledge of inorganic and organic chemistry, the medical student of the

present day studies inorganic, organic, analytical, and not infrequently physical chemistry, before admission to the first year of medical work. The proportionate number of hours devoted to biological chemistry during the first year of medicine indicates that this study is regarded as one of the most important of the medical sciences. Moreover the relative number of hours is being increased from time to time.

Biochemical Reactions.—All living things have been found to be composed of one or more cells. In consequence of this fact the cell has been taken as the unit of the organism. Chemically the cell is composed of such materials as proteins, carbohydrates, fats, phosphatides and cerebrosides; of extractive substances, such as creatin, creatinin, urea, amino acids and uric acid; and finally of inorganic salts, containing the cations, sodium, potassium, calcium, magnesium and iron and the anions, chloride, phosphate, carbonate, bicarbonate and sulphate. In many cases we find that these various constituents are chemically united with one another. Thus proteins often contain carbohydrate groups, the cerebrosides are complex fats which contain sugars and nitrogenous bases, and the proteins existing in protoplasm are always found to be united either with inorganic cations or anions. Of these compounds a certain number are crystalloids, while others, the proteins, phosphatides and higher carbohydrates, are known as colloids. The presence in plants and animals of compounds of colloidal nature, compounds which exist as gels and sols, which refuse to crystallize except in rare instances, and which are labile in nature and difficult to purify, has considerably increased the difficulties with which the biochemist is obliged to contend. However, the physical chemist and the colloid chemist have made notable progress in this field in recent years.

Chemical reactions are continually taking place within

the living cell. For the most part these reactions consist of oxidation, reduction, hydrolysis and dehydrolysis, or esterification, but a great many other reactions occur besides these. The sum of all of the reactions occurring in the cell is called metabolism. The oxidative reactions are included under the term catabolism and those reactions of a synthetic, or constructive nature are included under the term anabolism. Metabolism is always in part catabolic and in part anabolic. The essential difference between the plant and the animal is that the reactions of the former are to a large degree anabolic, while the reactions of the latter are, for the most part, catabolic. The plant cell containing chlorophyll has the power to synthesize carbohydrate from carbon dioxide and water, under the influence of sunlight, and to synthesize amino acids, when supplied in addition with such simple raw material as certain inorganic salts. Power to carry on synthetic reactions of this sort is not possessed by the animal cell. The animal cell is incapable of storing the energy of sunlight and is, therefore, obliged to depend either directly, as in the case of herbivora, or indirectly, as in the case of carnivora, upon the plant for its supply of energy, as well as for its supply of the majority of the amino acids. The animal does possess synthetic ability in certain directions, however, and to a very considerable degree. For example, any one of the cells from the liver can build and repair itself; can synthesize fibrinogen, glycogen, bile pigments and bile acids; can form urea from ammonium carbonate; can convert fructose and galactose into glucose; can convert glucose, or some degradation product of glucose, into fat; can detoxicate a great variety of poisonous substances; can form antitoxins; and can undoubtedly perform many other kinds of chemical work of a synthetic nature.

Methods of the Biological Chemist.—In attacking the complex and difficult problems that confront him the bio-

chemist is obliged to resort to a variety of methods of experimentation and to take the utmost advantage of all improvements and advances achieved in the different fields of chemistry, physics, biology and the medical sciences. In some cases the hospital is able to provide opportunity and material for study, in other instances animal experimentation has been found to be the best and indeed the only means of solving some problem. Of the numerous methods used by the experimentalist a few examples are:

1. Chemical analysis of plant and animal tissues and fluids.
2. Search for new and as yet undiscovered plant and animal compounds.
3. Study of metabolic processes.
4. Study of hormones and their action.
5. Perfusion of surviving organs.
6. Feeding animals certain chemical compounds and observing what end product has been eliminated in the urine.
7. Study of the mechanics of enzyme action.
8. Study of the regulatory mechanism by which the body preserves its constancy of composition and reaction.
9. Feeding experiments to establish the nutritive value and vitamin content of foods.

Besides working with the test tube and the pipette, the colorimeter, the centrifuge and the analytical balance, the biological chemist is not unwilling to make use of the microscope, the ultra-microscope, the spectroscope, the animal calorimeter and the x-ray tube. Even the moving-picture machine and the radio have been recently pressed into service.

Biochemical Literature.—Biochemical literature can be divided into four types: text books, monographs, original articles and abstracts.

Such text books as Hammarsten and Hedin's "Physio-

logical Chemistry," Mathews' "Physiological Chemistry" and Morse's "Applied Biochemistry" are recommended to students because these volumes give a very complete presentation of the subject and include many topics that have been omitted from the present book. The text books of Mathews and Morse contain laboratory experiments and analytical methods as well as lecture material. For laboratory manuals the student is especially advised to consult Hawk's "Practical Physiological Chemistry" and Folin's "Laboratory Manual of Physiological Chemistry." These two manuals contain a rather complete list of the analytical methods most frequently employed in the analysis of urine and blood.

The elementary student should not remain content with the study of text books, but should soon turn his attention to monographs. Fortunately there exists a large number of monographs dealing with a variety of biochemical topics. The monographs edited by Plimmer and Hopkins will be found especially helpful. A number of excellent monographs can be found in the "Physiological Reviews."

Original articles in English are to be found in "The Journal of Biological Chemistry," "The Journal of the American Chemical Society," "The Biochemical Journal," "The American Journal of Physiology," "The Journal of Physiology," "The Journal of General Physiology," "The British Journal of Experimental Biology," "The Journal of the American Medical Association" and other journals not included in this list. The two most important German periodicals are possibly: "Zeitschrift für physiologische Chemie" and "Biochemische Zeitschrift."

Abstracts are valuable largely for the purpose of finding out whether the original article is likely to contain matter of interest in connection with a certain topic. Abstracts of chemical and biological nature appear in the "Chemical Abstracts" of the American Chemical Society, in "The Pro-

ceedings of the Society for Experimental Biology and Medicine," in "Physiological Abstracts," and "Biological Abstracts." The index volumes of "Chemical Abstracts" will be found especially valuable for the beginner who does not know what journals to consult for articles bearing upon some specific topic.

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TEXTBOOK OF BIOLOGICAL CHEMISTRY

BIOLOGICAL CHEMISTRY

Chapter I

THE FATS

Introductory Remarks

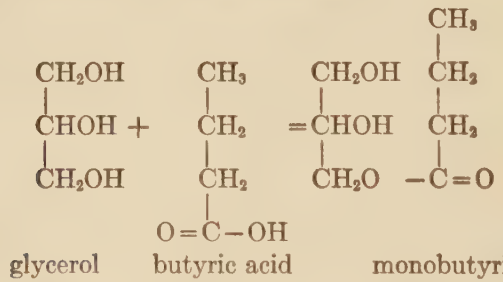
Distribution.—The fats comprise a class of organic compounds widely distributed in plants and animals. We find them in every living cell. In the cytoplasm droplets of fat are present, and in fat cells nearly the whole cell is composed of fat. No fat is present in the nucleus. In Man, according to Voit, about 20 per cent of the body weight consists of fat, and the proportion may be greater in a fat person. We find deposits of fat in the marrow of the bones, in the intermuscular connective tissue, in subcutaneous connective tissue and especially in the abdominal cavity where it is located about organs and is adherent to the mesentery and great omentum. In blood we find only traces of fat, except after a meal, when a considerable amount of fat may be present in a very finely emulsified form. In plants, fats are present in small quantities in the roots, branches and leaves, but are present in very large quantities as reserve foods in the seeds. Thus, Brazil nuts and pecans contain about 70 per cent of fat and almonds 54 per cent.

Terminology.—The terms oil and fat are synonymous, oils being fats which are liquid at ordinary temperatures. In using the word "oil," one should be careful to discriminate between the fixed oil or fat, the volatile oil or hydro-

carbon (gasoline, kerosene), and the essential oil (oil of turpentine, oil of wintergreen).

Properties of Fats.—Fats are greasy, white, or yellowish, especially when old, tasteless and odorless when fresh, but developing odor on becoming rancid, insoluble in water, soluble in ether, petroleum oils, chloroform and hot alcohol. Castor oil and linseed oil are soluble in cold alcohol. Fats from different sources have different melting points, on account of differences in composition.

Chemical Constitution.—In 1811 Chevreul showed that fats are glyceryl esters of the higher fatty acids. When one molecule of butyric acid combines with one molecule of glycerol, monobutyrin is formed with the elimination of water, the reaction being as follows:



glycerol butyric acid monobutyrin

Another molecule of butyric acid will give the compound dibutyrin, a third molecule of butyric acid gives tributyrin or, as it is called to-day, simply butyrin. We sometimes find small amounts of mono- and diglycerides in the natural fats, but most of the fat is composed of the triglycerides. These triglycerides are generally found to contain two, or even three different fatty acids in the same molecule, and are consequently spoken of as mixed triglycerides. Besides containing mono-, di- and triglycerides, the natural fats contain traces of free fatty acids, as well as waxes, sterols, phosphatides, cerebrosides, carotinoids and vitamins, which will be discussed later.

The Fatty Acids

There are at least six different series of fatty acids that are known to exist in the fats. These are:

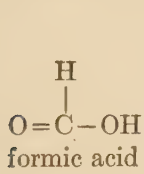
The acetic series.
The oleic series.
The linolic series.
The linolenic series.
The clupanodonic series.
The ricinoleic series.

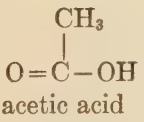
The acetic series of fatty acids is saturated; all of the others are unsaturated.

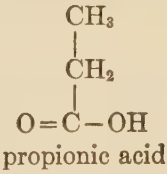
Acetic Series

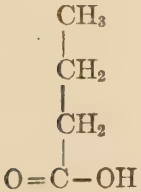
This is the best known and most important group of fatty acids. The series consists of saturated homologs of the general formula $C_nH_{2n}O_2$, each member of the series differing from the preceding member by CH_2.

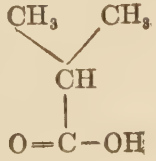
Of these fatty acids only the members with an even number of carbon atoms are usually found in fats. Valeric acid with 5 carbon atoms is an exception, being found in porpoise, dolphin and whale blubber. The structural formulas of the first five acids of the series together with isomeric forms of butyric and valeric are given below:

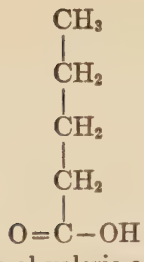

formic acid


acetic acid

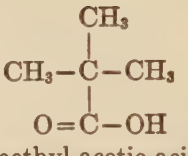

propionic acid


normal butyric acid

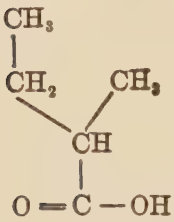

isobutyric acid



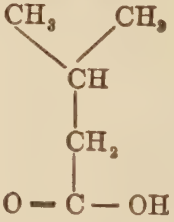
normal valeric acid



trimethyl acetic acid



active valeric acid



isovaleric acid

Nearly all of the higher members that occur in nature are straight-chain compounds. These are:

fatty acid	*number of carbon atoms*
caproic	6
caprylic	8
capric	10
lauric	12
myristic	14
palmitic	16
stearic	18
arachidic	20

The fatty acids with 7, 9, 11, 13, 15 and 17 carbon atoms do not occur in nature and therefore do not interest us. The names given to the fatty acids are often taken from the origin of the fat containing them. Thus, butyric acid is present in cow's butter, valeric in valerian root, caproic, caprylic and capric acids in goat's butter, lauric in laurel oil, myristic in nutmeg (*myristica*) oil, palmitic is in palm oil and stearic occurs in suet, the Greek for which is stear.

The two most important fatty acids in the acetic series are palmitic and stearic. These acids are found in nearly

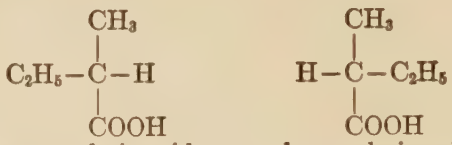
all fats. Coconut oil contains a number of the intermediate fatty acids and butter fat contains all of these acids, from butyric to stearic inclusive.

Properties of Acids of Acetic Series.—The lower members of the series are heavier than water, but as the molecular weight increases the greater relative content of hydrogen is accompanied by a decrease in specific gravity, which becomes less than 1. The first members of the series are rather strong acids, but the higher homologs are very weak acids. Again, formic, acetic and propionic acids have a strongly acid odor; butyric, valeric, caproic and caprylic have a rather disagreeable odor, but palmitic and stearic acids are nearly odorless. The fatty acids from formic to capric inclusive are liquids. The higher homologs are solids. The acids up to caproic are soluble in water, caproic itself being slightly soluble. Palmitic and stearic acids are very insoluble in water. With every CH_2 group added the boiling point of the acid increases 19° C.

Chain Isomerism.—Most of the naturally occurring fatty acids of the acetic series are straight-chain compounds, but acids with branched chains do exist, especially among those of high molecular weight, such as cerotic C_{26}, melissic C_{30} and the like. While we have but one possible formic, acetic, or propionic acid, we can have two butyric acids, the normal and the isobutyric (see table on page 3, and with valeric acid we find that four chain-isomers are possible (see table on page 4. With caproic acid, eight chain-isomers are possible and as one ascends the homologous series the possible number of chain-isomers increases with startling rapidity.

Stereo-isomerism.—Of the four possible valeric acids, only two occur in nature—isovaleric and active valeric acids. If we examine the structural formula of active valeric acid, we can see the possibility of another kind of isomerism, namely, optical, or stereoisomerism. The form-

ula of active valeric acid can be written in space in two different ways, giving a *dextro* modification and a *levo* modification:



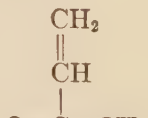
dextro valeric acid levo valeric acid

The two modifications are in the same relation to each other as an object and its mirror image, or as the right hand and the left hand. This is an instance of stereo-, or space isomerism, and is found in chemical compounds containing asymmetric carbon atoms, that is, carbon atoms whose four valences are satisfied by four different groups (compare formulas on page 4). Such compounds have two optically different modifications, similar in every way, except that one rotates the plane of polarized light to the left and the other rotates it to the right.

Active valeric acid exists as l. valeric acid, as d. valeric acid and as inactive, or i. valeric acid. When synthesized by the chemist the inactive form of valeric acid is always obtained. By proper means this can be resolved into its two component parts, the dextro and levo forms. Fats that contain optically active fatty acids are themselves optically active.

Oleic Series

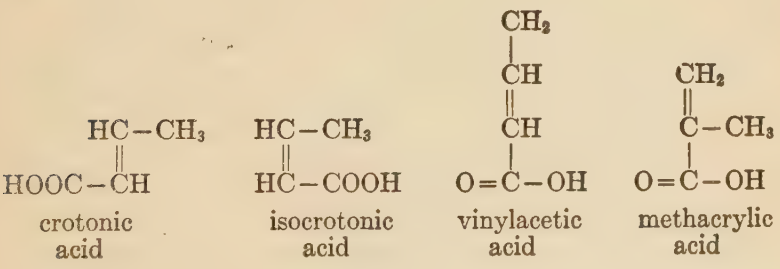
The fatty acids of the oleic series are unsaturated and have the general formula $C_nH_{2n-2}O_2$. Each acid contains one double bond. The simplest member of the series is acrylic acid, whose formula is:



acrylic acid

Cis-trans Isomerism.—It is found that acids of the oleic series exhibit what is known as cis-trans, or position isomerism. This is on account of the double bond. This type of isomerism offers two possible arrangements of the chain. In one of these arrangements, the cis, the chain is in the form of a C, in the other, the trans form, the chain is in the form of an S. The change from one form to the other can often be brought about by shaking the fat, or the fatty acid, with strong nitric acid, especially in the presence of a little mercury. In some cases fatty acids of the oleic series do not exhibit cis-trans isomerism. Possibly the reason for this is that the double bond is situated too near one end of the chain.

With four carbon atoms we have possible four unsaturated fatty acids, crotonic, isocrotonic, vinylacetic and methacrylic. Crotonic and isocrotonic acids are cis-trans isomers; it is not known which is the cis and which is the trans. The structural formulas of the four acids are given below:

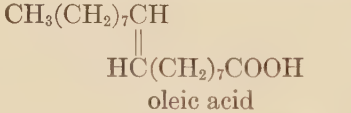


Isocrotonic acid is present in croton oil, which was formerly used as a purgative.

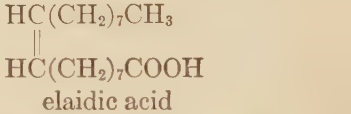
With five carbon atoms we have angelic acid, which is present in angelica root. The cis-trans isomer of angelic acid is tiglic acid, present in croton oil and oil of chamomile. Other members of oleic series are listed as follows:

fatty acid	*number of carbon atoms*
physetoleic	16
hypogaeic	16
oleic	18
erucic	22

The most important member of the oleic series, and indeed of any series of fatty acids, is oleic acid itself, containing 18 carbon atoms. Oleic acid is a liquid at ordinary temperatures, although stearic acid, with 18 carbon atoms, is a solid. This illustrates the effect of the double bond upon the melting point. When oleic acid is shaken with strong nitric acid and metallic mercury it is converted into the solid elaidic acid. According to Baruch, the liquid oleic acid is the trans isomer and the solid elaidic is the cis isomer. The structural formulas are given below:



oleic acid



elaidic acid

Oleic acid is present in nearly all fats and in particularly large proportions in many of the vegetable oils, such as olive oil and almond oil. The fat of cold-blooded animals contains a large amount of oleic acid, while the fat of warm-blooded animals contains relatively less oleic and relatively more palmitic and stearic acids. Oleic acid constitutes about 60 per cent of the fatty acids present in human fat. The composition of animal fat depends somewhat upon its location in the body; in general it can be said that adipose tissue, which contains storage fat, is more largely made up of saturated fatty acids than tissues in which fat is being oxidized rather than stored.

Because of the presence of a double bond, the fatty acids of the oleic series are more reactive chemically than those of the acetic series. Chlorine, bromine, iodine, hydrogen and oxygen are taken up at the point of unsaturation. Thus, upon adding bromine to oleic acid, two atoms of

bromine unite with the double bond, giving di-bromstearic acid:

$$\underset{\text{oleic acid}}{\begin{matrix}CH_3(CH_2)_7CH\\ \|\\ HC(CH_2)_7COOH\end{matrix}} + Br_2 = \underset{\text{di-bromstearic acid}}{\begin{matrix}CH_3(CH_2)_7CHBr\\ |\\ BrHC(CH_2)_7COOH\end{matrix}}$$

Iodine will not react with oleic acid unless a carrier is present. Mercuric chloride is used for this purpose and the solution containing potassium tri-iodide and mercuric chloride is known as Hübl's reagent. The oxidation of oleic acid results in the formation of di-hydroxystearic acid. The reaction is:

$$\underset{\text{oleic acid}}{\begin{matrix}CH_3(CH_2)_7CH\\ \|\\ HC(CH_2)_7COOH\end{matrix}} + H_2O + O = \underset{\text{di-hydroxystearic acid}}{\begin{matrix}CH_3(CH_2)_7CHOH\\ |\\ HOHC(CH_2)_7COOH\end{matrix}}$$

When oleic acid is treated with hydrogen gas in the presence of a catalyst, it is reduced to stearic acid. This reaction is of considerable commercial importance, for by means of it liquid fats can be converted into solid fats which are valuable in cooking. "Crisco" and "Snowdrift" are examples. The process is usually spoken of as the hydrogenation of fats. The catalyst which is used is finely divided nickel and the reaction takes place at about 200°C.

Linolic Series

A still more unsaturated series of fatty acids is the linolic, or linoleic series, with the general formula $C_nH_{2n-4}O_2$. Members of this series contain two double bonds and consequently can unite with four atoms of halogen. The only well known member of this series is linolic acid, which is present in most fats. Linolic acid contains 18 carbon atoms and upon reduction is converted into stearic acid. Linolic acid is present in linseed oil, whence the name.

Linolenic Series

Fatty acids of the linolenic series contain three double bonds and have the general formula $C_nH_{2n-6}O_2$. The important member of this family is linolenic acid, found in linseed oil.

Fats that contain acids of the linolic and linolenic series oxidize spontaneously, taking up oxygen from the air. As the process of oxidation proceeds, the fat becomes more viscous and may finally become solid; the fat is therefore popularly said to "dry." We can classify fats which do not contain acids of the linolic, or linolenic series as non-drying oils. Some examples are olive, almond and peanut oils. Fats showing a moderate ability to take up atmospheric oxygen are called semi-drying oils. Cottonseed and corn oils come under this classification. The drying oils are linseed oil, soy bean oil, walnut oil and Chinese wood, or tung oil, the latter being the most powerful drying oil known. On account of their ability to form a solid, elastic film upon drying these four oils find important application in painting.

Other Series of Fatty Acids

The clupanodonic series of fatty acids, with the general formula $C_nH_{2n-8}O_2$, is represented by clupanodonic acid, $C_{18}H_{28}O_2$, from Japanese sardine oil.

The ricinoleic series with the general formula $C_nH_{2n-2}O_3$ is represented by ricinoleic acid, which is the principal constituent of castor oil (*ricinus*). Ricinoleic acid contains a hydroxyl group, which is responsible for the cathartic action of castor oil. On account of the presence of the hydroxyl, castor oil will react with sulphuric acid to form a sulphonic acid. This compound is known as Turkey Red oil, and is used in dying. Castor oil is readily digested by

animals, but Gage has found that none of it is absorbed. The structural formula of ricinoleic acid is:

$$CH_3(CH_2)_5\ CHOHCH_2CH{=}CH(CH_2)_7COOH$$

When the sodium soap of ricinoleic acid is heated, secondary octyl alcohol, otherwise known as caprylic alcohol, is formed. This substance has found considerable use in biochemical laboratories for the prevention of foaming.

Hydrolysis of Fats

In the plant, or animal, the hydrolysis of fats is brought about by enzymes (see Chapter X). Enzymes that hydrolyse fats are known as lipases. These enzymes are found in the digestive tract and are also present in oily seeds. Reactions involving hydrolysis are of importance to the biochemist because the process of digestion consists of a hydrolysis of the food. We can define "hydrolysis" as the splitting of a chemical compound with the taking up of water. In the laboratory fats are hydrolysed, or saponified, by boiling with water containing bases, e.g., sodium hydroxide, calcium hydroxide, lead oxide; acids, e.g., sulphonic acids, or by means of superheated steam alone. The most efficient reagent is alcoholic potash, or alcoholic soda. Its efficiency is due to the solvent action of the alcohol upon the fat, which results in a greater amount of contact with the alkali.

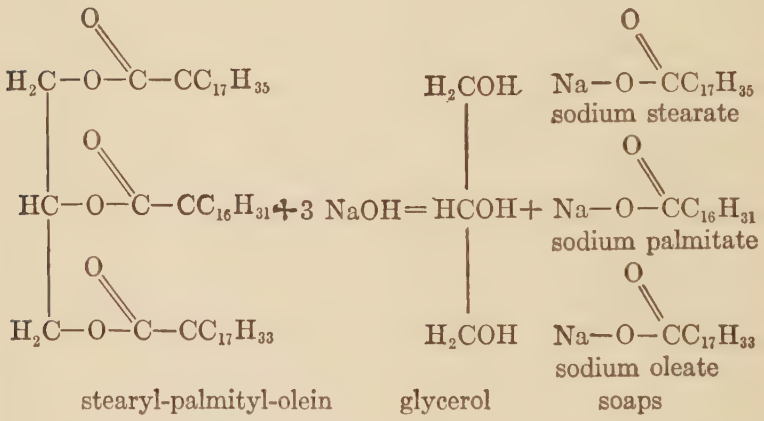
Rancidity

When fat is kept at room temperature, it slowly undergoes hydrolysis with the liberation of fatty acids. At the same time oxidation occurs, some of the glycerol being oxidized, and some of the fatty acids being oxidized, with the formation of aldehydes, ketones and fatty acids of low molecular weight. The product has a disagreeable odor and an acid reaction.

Rancidity is probably caused by enzymes and bacteria in the presence of moisture, light and oxygen. When a rancid fat is melted and shaken with a few drops of Congo red solution the indicator is turned blue by the free fatty acids.

Soaps

Formation.—When fats are hydrolysed by boiling with metallic hydroxides, glycerol and soaps are formed. This reaction is illustrated by the following typical example:



We accordingly define a soap as a metallic salt of one of the higher fatty acids.

Soluble and Insoluble Soaps.—The ammonium and alkali soaps are soluble in water, but soaps of the alkaline earths and of the heavy metals are insoluble in water, although soluble in oil. The lead salt of oleic acid possesses the distinction of being soluble in ether, while lead palmitate and lead stearate are only sparingly soluble. The most characteristic properties of the water-soluble soaps are their ability to form suds, their alkaline reaction when dissolved in water, and the formation of a precipitate on the addition

of calcium and magnesium salts. If we examine the alkali salts of the lowest fatty acids, such as acetic, or butyric, we fail to find soap characteristics. Thus, sodium acetate has none of the properties of a soap and bears more resemblance to sodium chloride. Soap character is first exhibited in the acetic series, by salts of lauric acid. Salts of the higher fatty acids do not exist as crystalloids in aqueous solution, but as colloids. For example, an aqueous solution of sodium stearate contains colloidal particles which are possibly composed of stearate ions and stearic acid molecules.

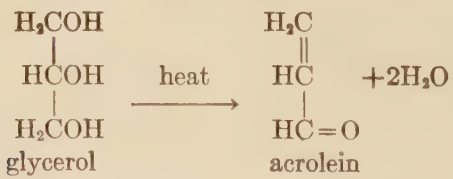
Hydrolysis of Soap by Water.—When soap is dissolved in water, hydrolysis takes place, with the formation of sodium hydroxide and fatty acid. This hydrolysis is prevented by the addition of alcohol, or glycerol to 50 per cent concentration. In a solution containing 50 per cent, or more, of alcohol, soap behaves as a crystalloid. The presence of free sodium hydroxide in an aqueous solution of soap can be shown by adding a drop of phenolphthalein, which colors the solution red. If one now adds an equal volume of alcohol, the red color disappears unless the soap originally contained free alkali. The presence of an excess of alkali in soap can be shown by treating dry soap with phenolphthalein. Owing to the hydrolysis of soap by water, it is impossible to titrate an aqueous solution of the higher fatty acids with standard alkali, but if at least 50 per cent of alcohol is present the titration is perfectly satisfactory.

Emulsions.—In common with all these substances classified as hydrophilic colloids, soap solutions possess the power of emulsifying immiscible liquids. If we vigorously shake a mixture of oil and water a temporary emulsion will form, but on standing the two phases soon separate. If a sufficient amount of soap is present, a rather stable emulsion will be formed. We can consider the oil droplets to be

covered with a protecting envelope of colloidal soap. This envelope keeps the minute droplets from coalescing. The soaps of the alkalis produce emulsions of oil in water; on the other hand, the soaps of the alkaline earths cause the formation of emulsions of water in oil. Stable, though dilute, emulsions of oil in water can be obtained by dissolving the oil in hot alcohol and pouring the solution into a large volume of water. In this instance no emulsifying agent is present.

Analysis and Identification of Fats

Fat can be recognized by its ability to dissolve in ether, or chloroform, and by its ability to form soap on hydrolysis. One of the commonest tests for fat depends upon the presence of glycerol in the fat molecule. When a fat is heated to a high temperature the glycerol decomposes with the formation of acrolein, which is recognized by its irritant effect upon the eyes and nose. In the presence of inorganic salts this reaction proceeds at a lower temperature and it is, therefore, usual to perform the test by heating the fat with potassium bisulphate. For the sake of simplicity the reaction is usually written, starting with free glycerol:



A much more satisfactory test for glycerol is that of Mandel and Neuberg. This test consists in oxidizing the glycerol by sodium hypochlorite to dihydroxy acetone. Dihydroxy acetone reacts with orcinol and concentrated hydrochloric acid to give a violet color.

Histological tests for fat are made with fat-soluble dyes,

such as Sudan III, or with osmic acid, which stains the fat black. The black stain is due to the reduction of the osmic acid to a lower oxide by the unsaturated fatty acids present in the fat.

The natural fats are complex mixtures and cannot be analysed as are many individual chemical compounds. Fats obtained from different species of plants and animals possess certain rather constant characteristics by means of which they can be identified. These properties depend for the most part upon the molecular weight and degree of unsaturation of the fatty acids that are present. Certain of the more important physical and chemical characteristics that are used for the identification of fats are explained below:

melting point—the temperature at which the fat changes from the solid to the liquid state.

iodine number—the number of milligrams of iodine taken up by 1 gram of fat from a solution of iodine, containing mercuric chloride as a carrier.

saponification number—the number of milligrams of potassium hydroxide required for the complete hydrolysis of 1 gram of fat.

Reichert-Meissl number—the number of cubic centimeters of tenth normal alkali required to neutralize the total volatile fatty acids set free and distilled in a current of steam from 5 grams of fat, according to a standardized procedure.

Hehner number—the percentage of fatty acids insoluble in hot water.

The numerical expressions of these characters for ten familiar fats are given in the accompanying table. The student should study particularly the figures which have been italicized and those in column 2, and explain them on the basis of the chemical composition of the fat, or wax.

TABLE OF CHEMICAL AND PHYSICAL CONSTANTS SERVING TO DISTINGUISH SOME COMMON FATS

oil, fat, or wax	1. melting point	2. iodine number	3. sapon. number	4. Reichert-Meissl number	5. Hehner number
butter	*28–36*°C.	28–42	*216–241*	*24–34*	*85–90*
beef fat	42–50	25–41	196	0.3–0.5	96–96.5
lard	36–46	48–64	195–203	0.2–0.8	93–95
cocoa butter	28–35	32–39	192–195	0.2–0.9	94.5
lard oil	liquid at ordinary temperatures	67–82	193–198	.....	97
olive oil		77–95	185–196	0.6	95–96
cottonseed oil		104–116	191–196	0.7–0.9	95–96
linseed oil		*175–210*	190–201	.....	95–95.5
cod-liver oil		154–170	179–190	0.2	95.5
beeswax	62–66	8.5–11	88–98	.....	.....

WAXES

Associated with the fat present in animals and plants, we always find small amounts of waxes. In some cases large amounts of wax are present. A wax may be defined chemically as an ester of one of the higher fatty acids and a monatomic alcohol. The waxes are usually more difficult to hydrolyse than the fats and after hydrolysis, the complex alcohol which is liberated remains undissolved. Waxes are used by the plant as a protective covering. In the animal it is believed that fatty acids are, to a certain extent, transported as waxes (Bloor).

Some examples of animal waxes are: cetyl palmitate, which is found in spermaceti ($C_{16}H_{35}OH$ cetyl alcohol), and

myricyl palmitate, one of the constituents of beeswax ($C_{30}H_{61}OH$ myricyl alcohol). Lanolin, or wool wax is composed of esters of cholesterol and iso-cholesterol with hydroxy acids of unknown constitution ($C_{27}H_{45}OH$ cholesterol).

Cholesterol.—Cholesterol is a complex monatomic alcohol of considerable importance. Its structural formula is not certain, but cholesterol can be said to belong to the hydroaromatics, or terpenes. Cholesterol occurs both in the free state and as a constituent of waxes in nearly every animal cell and in the blood stream as well. Gall stones are sometimes found to be composed almost entirely of free cholesterol. Considerable quantities of cholesterol are present in the central nervous system and can be extracted by means of acetone. Cholesterol is easily identified by many beautiful color reactions. When treated with dry chloroform, acetic anhydride and a drop of concentrated sulphuric acid, a grass green color is produced. This reaction is used as the basis for the estimation of cholesterol in blood. Normal blood contains from 140 to 180 mg. of cholesterol per 100 cc. In pernicious anemia low values are found; in diabetes, nephrosis, and especially in lipemia and cholelithiasis, high values are found.

Beyond the fact that cholesterol prevents the hemolysis of red blood corpuscles and possibly serves for the transportation of fatty acids, we know little of its biological significance, but its wide distribution in animal tissues attests to its importance. The rapid dissolution of the red blood corpuscles in pernicious anemia has been assumed by some to have a connection with the low blood cholesterol in this disease.

In plant fats one finds instead of cholesterol, the isomeric substance phytosterol. Phytosterol can be differentiated from cholesterol by determining the melting points of the acetates.

Carotinoids

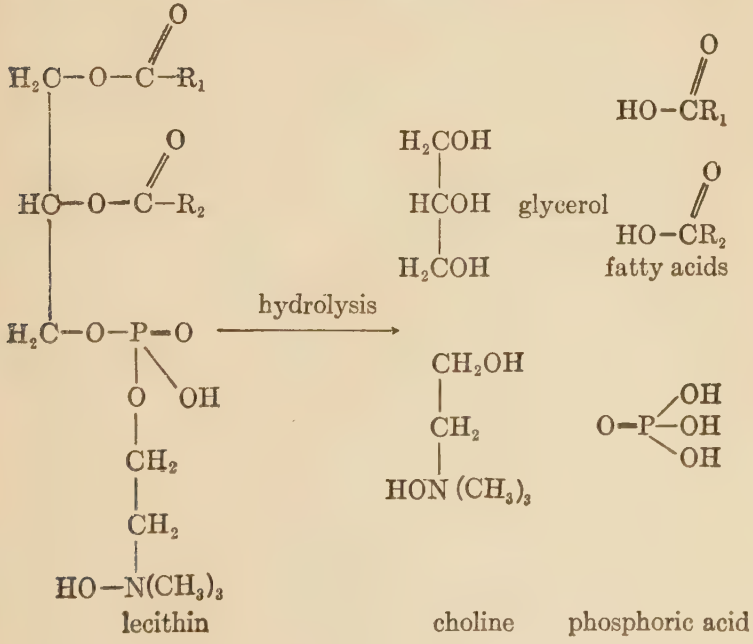
The natural fats also contain minute amounts of yellow and orange colored substances the significance of which is quite unknown. They are known as carotinoids; examples are carotin and xanthophyll. Carotin derives its name from the carrot, which contains it. Carotin is also found in the blood serum of certain animal species and in the corpus luteum. It is present in the fat of the cow, the horse and man. It is not present in the dog, cat, rabbit, pig or sheep. Carotin is a hydrocarbon, with the formula $C_{40}H_{56}$. Xanthophyll, $C_{40}H_{56}O_2$, is closely related to carotin chemically. Together with carotin it occurs in most green leaves. It is present in hen's fat and in the yolks of eggs. When hens are fed on diets containing little or no xanthophyll, the yolks of the eggs eventually lose nearly all of their yellow color. The carotinoids are frequently found to be associated with one of the vitamins, but do not appear to be of value for the purpose of nutrition.

Phosphatides

Besides containing waxes and carotinoids, the natural fats have been found to contain still another class of substances, the phosphatides, or phosphorized fats. These have been called lipoids by Overton, and phospho-lipins by Leathes. The phosphatides are present in all living cells and especially in nervous tissue. In storage fat only small amounts of phosphatides are found, but the fat present in an organ such as the liver may consist very largely of phosphatides. Phosphatides are soluble in alcohol, ether and chloroform and will usually form colloidal solutions with water.

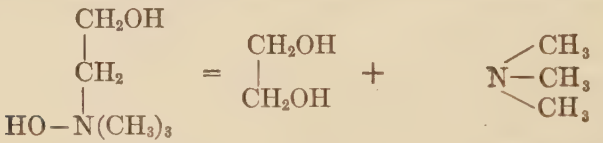
Lecithin.—The phosphatides that have been most studied are lecithin and cephalin. Lecithin can be readily prepared from egg yolk, which contains 9 per cent, by extracting the lecithin with alcohol and precipitating with acetone.

Lecithin obtained in this way is a waxy, yellowish solid which forms opalescent, soapy solutions with water. On contact with the air, lecithin slowly takes up oxygen and deepens in color. The structural formula of lecithin is given below, and shows it to be a fat which has one fatty acid molecule replaced by phosphoric acid, the phosphoric being also attached to the nitrogenous base, choline. It will be seen that lecithin contains an asymmetric carbon atom and is therefore capable of optical isomerism. The fatty acids in lecithin may vary, giving different lecithins. Levene has found stearic, palmitic and oleic acids in the lecithin from hen's egg yolk, therefore at least two different lecithins must be present in egg yolk. Lecithin from the liver has been found to contain arachidonic acid, which belongs to the clupanodonic series of fatty acids. Lecithin can be hydrolysed by the same catalysts (including enzymes) that hydrolyse the fats. The products of hydrol-



ysis are: fatty acids, glycerol, phosphoric acid and choline. Glycerophosphoric acid is an intermediate product of the hydrolysis.

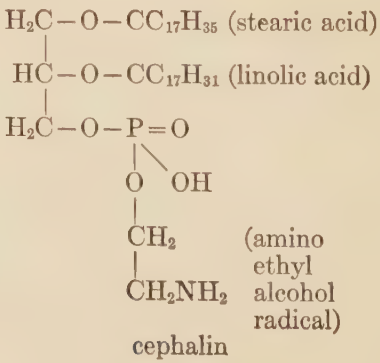
Choline.—Choline, a product of the hydrolysis, occurs in the free state in bile and blood in small amounts. It is present in herring brine, in hops and in mustard seeds. Upon heating either lecithin, or choline with concentrated sodium hydroxide, the choline is decomposed into glycol and tri-methyl amine. Tri-methyl amine is easily recognized on account of its fishy odor. The reaction is:



choline glycol tri-methyl amine

Choline markedly stimulates the contractions of the small intestine. In this action it is opposed by adrenalin, which inhibits the contractions. With concentrated solutions of iodine in potassium iodide, choline forms a per-iodide, which gives characteristic crystals. This is a delicate test for choline.

Cephalin.—Cephalin is another important phosphatide. It is of general occurrence, but is usually obtained from the brain. Levene has assigned to cephalin the following structural formula:



cephalin

Cephalin is thought to be one of the factors necessary for the coagulation of the blood and is probably present in the blood platelets. The jack bean contains a phosphatide having a similar action on the clotting of blood. Phosphatides are present in plants as well as in animals, but have been little studied. Levene has recently isolated both lecithin and cephalin from the soy bean.

Sphingomyelin.—Sphingomyelin is a phosphatide which is found in animal cells. It contains no glycerol, but is composed of the bases sphingosine and choline united with phosphoric acid, lignoceric acid and what is probably hydroxystearic acid.

Little is known of the physiological significance of the phosphatides. Leathes has suggested that fats are changed in the liver into phosphatides before they are oxidized.

Cerebrosides

The brain contains *phrenosin* and *kerasin,* which differ from the phosphatides in that they contain neither phosphoric acid nor glycerol. Both of these substances contain the sugar galactose and the complex nitrogenous base sphingosin. Phrenosin contains in addition phrenosinic acid, while kerasin contains lignoceric acid. The cerebrosides can be classified as galactosides. It appears that the presence of lactose (which contains galactose) in milk is necessary for the synthesis of these substances by the young mammal.

The Digestion of Fats

The average person consumes about 50 grams of fat per day. As each gram of fat that is oxidized gives 9.4 large calories, it can be seen that fats are valuable for the production of energy. The high fuel value of fats is due to their high content of carbon and hydrogen and to their low proportion of combined oxygen. One gram of coal, upon

oxidation, gives about 8 calories, while one gram of radium, in its decomposition to helium and lead, gives 10,000,000 calories.

Gastric Digestion of Fat

The presence of a fat-splitting enzyme in the stomach—gastric lipase—was discovered by Ogata and later was rediscovered by Volhard. This enzyme is present in the gastric juice and is able to hydrolyse emulsified fat, such as is found in cream and egg yolk, with the formation of glycerol and fatty acids. As gastric lipase is killed, or inhibited by the action of strong acids, it seems unlikely that this enzyme performs much fat hydrolysis, owing to the presence of hydrochloric acid in the gastric juice. Some fat digestion undoubtedly takes place in the fundic part of the stomach, which is not acid during the early stages of gastric digestion.

Intestinal Digestion

The acid chyme upon reaching the duodenum is mixed with three secretions, the succus entericus, or intestinal juice, the pancreatic juice and the bile. These three digestive juices contain sodium bicarbonate, which neutralizes the hydrochloric acid present in the acid chyme. While the upper portion of the small intestine is usually acid, the middle and lower portions are usually neutral, or faintly alkaline, but contrary to the ideas formerly held, the contents of the small intestine do not become strongly alkaline. The reaction of the jejunum has been observed to reach the pH of 8.0 occasionally. The pancreatic juice contains the important fat-splitting enzyme steapsin. The action of steapsin on fats is aided by the bile salts, sodium glycocholate and sodium taurocholate, which function as coenzymes. The hydrolysis of fats is also greatly aided by the emulsification of the fat, a process which is brought

about by the churning movements of the intestine and by the presence of such colloidal substances as the bile acids and the proteins of the digestive juices. It can be readily seen that finely emulsified fat exposes much more surface to a digestive enzyme than does fat that is present in large globules. Fats that have high melting points are less readily digested than fats with low melting points; this difference may be due to a difference in the ease of emulsification.

Absorption of Fat

Fat is absorbed from the middle and lower portions of the small intestines and passes into the lacteals, where it can be readily seen to be present in a finely emulsified condition, and can be shown by chemical tests to consist of neutral fat. From the lacteals the fat passes into the

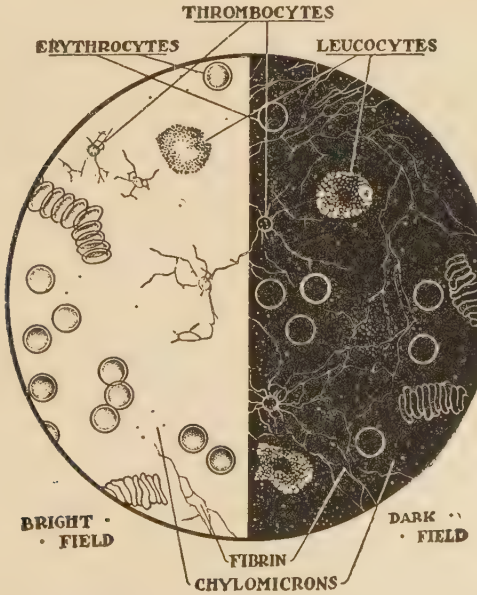


Fig. 1. Chylomicrons under dark-field microscope. (*From* Gage: The Microscope.)

receptaculum chyli and through the thoracic duct, into the left innominate vein at the junction of the left subclavian vein with the left internal jugular vein. The circulation carries the particles of finely emulsified fat (chylomicrons) to all parts of the body. From three to five hours after ingestion, the fat begins to be deposited in the mesentery, about the kidneys, and in other places of fat deposit (Gage). Fats that have been dyed, or halogenated, can be traced and found to be laid down in this manner. Figure I shows how Gage has been able to demonstrate chylomicrons in the blood by means of the dark field microscope. The blood stream serves merely to transport the emulsified fat to the tissues and after doing so retains only small amounts.

The manner in which fat is transported through the walls of the intestine is difficult to explain. We believe that one of the purposes of digestion is to make food material soluble, but the fatty acids that are formed by the hydrolysis of fat are just as insoluble as the fats themselves, hence it appears likely that the fatty acids are changed into soaps. Pflüger has advanced the doctrine of no absorption without digestion, and the importance of the steapsin of the pancreatic juice can be demonstrated by ligature of the pancreatic ducts. When this is done, fat absorption is greatly impaired. Tying off the bile duct also interferes with the absorption of fats. In cases with pancreatic tumors and in obstruction of the bile duct, the amount of fat in the feces is greatly increased in amount. This indicates the importance of both pancreatic juice and bile for the digestion and absorption of fat.

At the present time the most likely explanation of the mechanism of fat absorption is based on the assumption that the fatty acids formed by hydrolysis are brought into colloidal solution by the bile salts as alkali soaps and that after transportation into the intestinal epithelium they are resynthesized into neutral fat.

A less probable explanation of fat absorption is that the fat is so finely emulsified that the suspended particles can pass directly through the intestinal epithelium. Shaefer believes that the leucocytes, which are present in the intestinal epithelium in large numbers during digestion, serve to transport fat.

Of the fat absorbed from the small intestine only from 60 to 80 per cent can be recovered from the thoracic duct. Hence it seems possible that some fat may be absorbed directly into the capillaries surrounding the intestine. Of the fat ingested with the food, about 95 per cent is absorbed and about 5 per cent is excreted in the feces as calcium and magnesium soaps.

Metabolism of Fat

Deposition of Foreign Fats

Experiments have shown that iodized and dyed fats are deposited in the tissues after absorption. From this evidence one would expect that the fatty tissue of a fat-eating animal would have the same approximate composition as the food fat. But this is not the case. Each species of animal normally deposits fat of a characteristic quality. This selective deposition of fat may be due to the fact that the animal burns the sort of fat which it does not store, or that the animal may store fat which it has formed synthetically. It must be remembered that the nature of the fat varies with its location in the body, so that a type of fat not desirable for storage about the kidney might be suitable for storage in the liver. It has been found that the fat of milk is influenced by the nature of the food fat and that when cows are fed on cottonseed meal the butter has a low melting point.

The Synthesis of Fat

Liebig suggested that animals were capable of synthesizing fats from carbohydrates, but this view was abandoned when Voit and Pettenkofer brought forward experimental evidence to show that when dogs are fed on lean meat they store up a considerable amount of carbon, presumably as the carbon of fat. Other examples of a synthesis of fat from protein were supposed to be the formation of milk fat and the formation of fat in the so-called fatty degeneration of the liver and of nervous tissue. Some twenty years later Pflüger recalculated the results of Voit and Pettenkofer and showed that these investigators had taken too high a value for the carbon present in lean meat and too low a value for the nitrogen, and that the recalculated results showed only a slight storage of fat in some cases and no storage at all in others.

Physiologists now returned to the original view that fats can be synthesized by animals from carbohydrates. It was conclusively shown by Lawes and Gilbert, Soxhlet, Meissl and Strohmer and several others that pigs, geese and dogs, when fed on carbohydrate-rich food, are able to store considerable amounts of carbon in their bodies and that this carbon is in the form of fat. In these experiments the amounts of carbon and nitrogen ingested were obtained by analysing and weighing the food and the excretion of carbon and nitrogen was estimated by collecting and analysing the urine, feces and expired carbon dioxide.

To-day it is accepted without question that animals synthesize fat from carbohydrate. It has been shown that ripening nuts contain carbohydrates. As the process of ripening proceeds, the carbohydrates disappear and fats take their place. When the nuts undergo germination, this process is reversed. This last reaction has not been shown

to occur in the animal. The formation of some of the lower fatty acids from the sugars by certain bacteria is well known. Besides lactic and butyric acids, small amounts of caproic and caprylic acids have been observed to be produced. The manner in which the animal reduces carbohydrates to fats is not understood. It seems probable that some simple degradation product of glucose is used and that the fat is constructed by the addition of two carbon atoms at a time, since the fats that occur in nature nearly all contain an even number of carbon atoms. The synthesis of fat in animals does not take place unless the liver contains a large amount of glycogen.

By experiments upon diabetic dogs, Lusk and others have shown that animals are able to synthesize sugar from protein. As sugar can be used by the animal for the synthesis of fat, it appears that fat might be produced indirectly from protein, with the intermediate formation of carbohydrate. It is unlikely, however, that a human being could consume enough meat to lead to a synthesis of fat, although a carnivorous animal might do so. Since the animal is capable of synthesizing fat from carbohydrate one might expect that fat need not be present in the diet, but the Germans suffered from a kind of edema during the war that was believed to be due to a lack of fat. This disease may have been due to the lack of the fat-soluble vitamins, rather than to the lack of fat itself (see Chapter V).

Catabolism of Fat

The organism produces energy from fats by oxidation. In this process water and carbon dioxide are the end products. Fats are presumably oxidized in all living cells. The process of oxidation undoubtedly takes place by a series of successive chemical reactions, with the formation of a number of intermediate products. In a normal animal the

oxidation of fat goes to completion and no intermediate products of oxidation are apparent. In severe diabetes the oxidation of fat is imperfect and the products of this imperfect oxidation have served as a clew in the study of the reactions that are involved.

Mechanism of Oxidation.—In 1895 Knoop proposed his hypothesis of the beta-oxidation of fatty acids. This hypothesis assumes that the fatty acid is oxidized at the beta position (the second one from the carboxyl group), two carbon atoms being split off, and a lower fatty acid (one containing fewer carbon atoms) being formed. The whole process is then repeated until the molecule of fatty acid has become entirely catabolized.

Fatty acids occurring in the natural fats contain an even number of carbon atoms. Therefore, the process just described leads eventually to the formation of butyric acid (4 carbon atoms). This is oxidized completely under normal conditions, and forms carbon dioxide and water without much evidence of the intermediate products, but intermediate products can be found when the fat catabolism is abnormal. The theory of beta oxidation is supported by:

1. Knoop's experiments.

2. The presence of acetone bodies in animals in which the catabolism of fat is abnormal.

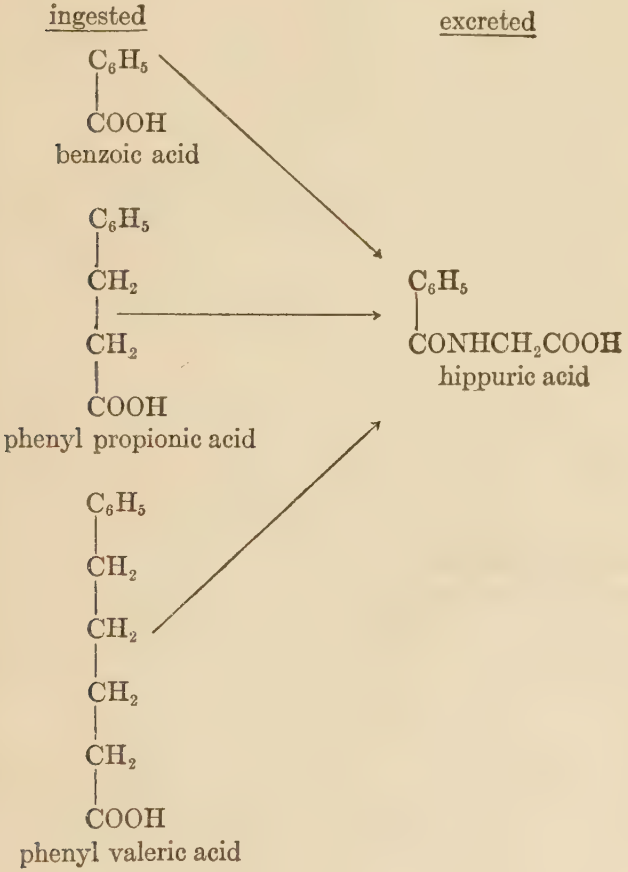
3. Dakin's investigation of the action of hydrogen peroxide on butyric acid.

4. Perfusion of the surviving liver with solutions containing certain fatty acids.

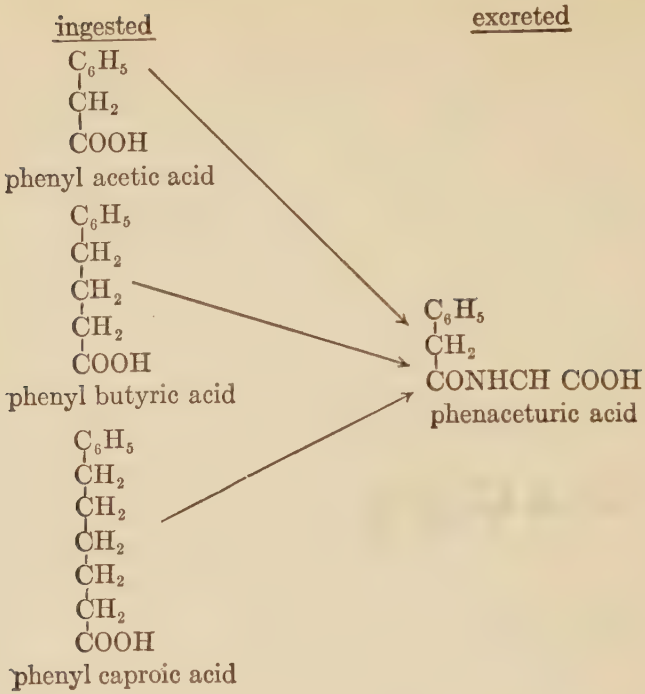
Knoop's Experiment

Knoop fed to dogs fatty acids having attached the benzene ring. As the benzene ring is usually difficult for the animal to oxidize, the process of catabolism could not be

carried out completely and certain products of oxidation could be isolated from the urine. When the dogs were given benzoic, phenyl propionic, or phenyl valeric acids, hippuric acid was always found in the urine:



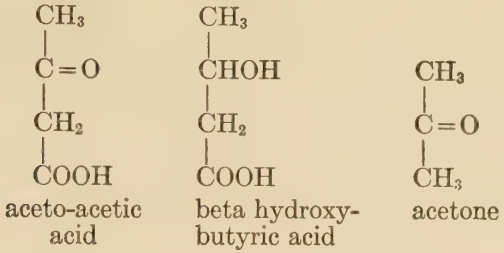
When phenyl acetic, phenyl butyric, or phenyl caproic acids were fed, phenaceturic acid was always found in the urine:



Note: The benzoic acid and phenyl acetic acid fed, or formed, in Knoop's experiment are harmful to the animal, but the cells combine them with glycocoll to form the non-toxic hippuric and phenaceturic acids respectively, which are eliminated in the urine.

Abnormal Fat Catabolism.—In the normal animal, as has been pointed out, the oxidation of fat proceeds smoothly and completely and no more than minute traces of intermediate products can be isolated. On the contrary, when animals are suffering from severe diabetes, the oxidation of fat is incomplete and very large amounts of the products of this incomplete oxidation accumulate in the blood and tissues, being found also in the urine. These

substances are called the acetone bodies. Their structural formulas are given below:



Aceto-acetic acid is believed to be the first of these acetone bodies to be formed, beta hydroxybutyric acid being a reduction product, and acetone being produced by the decarboxylation of the aceto-acetic acid. The acetone bodies appear when the animal is unable to oxidize carbohydrate (diabetes mellitus), or when the animal has very little carbohydrate to oxidize (starvation). During starvation the acetone bodies are produced in considerable amount, but in a normal person the quantity produced is not dangerous. It appears that the oxidation of sugar assists in some unknown manner the last stages of the oxidation of fats. In other words, it can be said that "fats burn in the flame of the carbohydrates." Aceto-acetic acid is believed to be one of the normal intermediate products of fat oxidation, but in the normal animal it is destroyed by oxidation as rapidly as it is formed.

Oxidation of Butyric Acid in Vitro.—Upon oxidizing butyric acid by means of hydrogen peroxide, Dakin found aceto-acetic acid and acetone among the products of the reaction, which proved that the formation of these substances in the catabolism of fat is possible.

Perfusion of the Surviving Liver.—Embden and Marx perfused surviving livers (taken from an animal immediately after death) with blood to which had been added

the ammonium salts of various fatty acids of the acetic series. They found that when butyric, caproic, caprylic and capric acids were added to the blood acetone was formed, but when fatty acids with an odd number of carbon atoms, such as valeric, heptoic and nonylic were used, acetone was not formed.

Antiketogenesis.—Shaffer has found that glucose, fructose, glycerol and similar substances greatly hasten the oxidation of aceto-acetic acid by hydrogen peroxide in vitro. This effect is entirely analogous to the action of these compounds in preventing the appearance of acetone bodies in a starving animal. Shaffer has called this action of the sugars and of glycerol "antiketogenesis." He has calculated that one molecule of glucose must be available for oxidation for every two molecules of aceto-acetic acid. When this ratio obtains there will be no appearance of the acetone bodies. It is possible to calculate approximately how much carbohydrate must be added to the diet to prevent the formation of acetone bodies. In this calculation the glycerol of the fat is counted among the antiketogenic substances and 62 per cent of the protein is considered to be capable of conversion to sugar, while about 15 per cent of the protein is convertible into aceto-acetic acid.

Summary

The apparent course of the oxidation of fats suggests—conclusive evidence is lacking—that the fatty acids resulting from the hydrolysis of fat are oxidized, a beta keto-acid being formed first. Hydrolysis of this keto-acid then possibly follows, the two lower carbon atoms being split off in the form of acetic acid, which is oxidized to carbon dioxide and water. The carbonyl group is changed to a carboxyl group, giving a new fatty acid with two carbon atoms less than the original acid.

It is possible, however, that the keto-acid is oxidized

instead of being hydrolysed and that the two carbon atoms that are split off form glyoxylic acid, which is then oxidized to carbon dioxide and water.

The processes of oxidation and hydrolysis, or oxidation and oxidation, are repeated, causing the fatty acid to lose two carbon atoms at a time until finally butyric acid is formed. The oxidation of butyric acid forms aceto-acetic acid. In the normal animal the aceto-acetic acid is oxidized to carbon dioxide and water, but the diabetic animal cannot oxidize aceto-acetic acid. The diabetic animal reduces a part of the aceto-acetic acid to beta hydroxybutyric acid and decomposes a part to acetone, all three of these substances being abnormal end-products of the catabolism of fat.

Since each molecule of fatty acid can furnish only one molecule of aceto-acetic acid, it can be seen that, weight for weight, the diabetic animal will form larger amounts of acetone bodies from fats containing fatty acids of low molecular weight than from fats containing fatty acids of high molecular weight. It is also apparent that the fatty acids with an odd number of carbon atoms cannot give rise to acetone bodies by beta oxidation. In order to take advantage of this fact, margarin, containing the 17 carbon atom margaric acid, has been prepared and has been used by Kahn in the treatment of diabetes. In diabetes the ordinary fats give rise to the acetone bodies, which are poisonous. Margarin, which goes by the trade name of "intarvin," does not lead to the formation of acetone bodies.

Chapter II

CARBOHYDRATES

Introduction

Carbohydrates comprise a second class of organic compounds occurring in plants and animals. Over 70 per cent of the food material in our diet is carbohydrate. In animal matter we find present but little carbohydrates, but the plant is largely composed of carbohydrate material. Carbohydrates are found in all living cells. Sometimes they are present in chemical combination with proteins (glycoproteins and nucleoproteins) and sometimes with lipins (glycolipins or cerebrosides).

The carbohydrates are composed of the elements carbon, hydrogen, and oxygen, and nearly all of them contain two parts of hydrogen to one of oxygen, whence the name carbohydrate. An exception to this rule is found in the methyl pentoses.

Carbohydrates can be divided into two groups, the sugars and the polysaccharides. The sugars are classified as mono-, di-, and tri- saccharides, disaccharides being composed of two monosaccharide molecules and trisaccharides being composed of three such molecules.

MONOSACCHARIDES

Classification

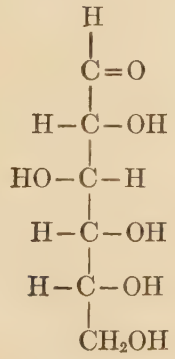
Monosaccharides are classified according to the number of carbon atoms they contain. Thus, the tetroses contain four, the pentoses, five, and the hexoses, six carbon atoms.

Monosaccharides are also classified, on the basis of their chemical constitution, into those containing an aldehyde group (aldoses), and those containing a ketone group (ketoses).

Aldohexoses

The most important of the monosaccharides are those sugars with six carbon atoms and an aldehyde group—the aldohexoses. The empirical formula representing the aldohexoses is $C_6H_{12}O_6$, or structurally:

Examination of this structural formula shows that four asymmetric carbon atoms are present (see page 6). Using the formula of Van't Hoff, that the number of stereoisomers=2^n, when n represents the number of asymmetric carbon atoms present in the molecule, we can calculate that sixteen different stereoisomeric sugars having the above formula are possible. Of these sixteen possibilities only three occur in nature: d. glucose, d. mannose, and d. galactose; and all but two of the sixteen have been prepared synthetically by Emil Fischer and others. d. Glucose has the structural formula:

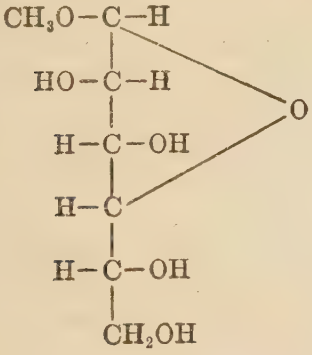


If we designate the occurrence of the hydroxyl attached to an asymmetric carbon atom by a + when it lies on the right side and by a − when it lies on the left side, we need only remember the formula + − − + +, counting from the aldehyde group, in order to be able to reproduce the stereoisomeric structure of d. glucose. The enantiomorph, l. glucose can be represented as − + − −, d. mannose as − − + +, and d. galactose as + − − +. It is evident that further combinations of the + and − signs can be arranged to represent each member of the sixteen possible aldo-hexoses, eight of the sixteen being optical antipodes of the other eight.

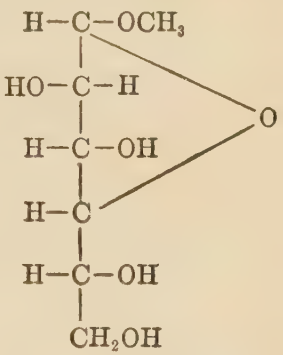
d. Glucose (dextrose, or grape sugar) is the most important of all sugars. It is almost universally present in plant and animal cells. The greater part of our carbohydrate food is absorbed as d. glucose. Glycogen, starch, and cellulose are anhydrides of d. glucose. The disaccharides, sucrose, lactose, and maltose contain d. glucose. d. Glucose is a white solid, forming minute crystals when pure. It is often seen in commerce as a thick syrup, or when solid in the form of granules. It is about one-third as sweet as cane sugar, easily soluble in water, and readily fermented by yeast. Glucose is capable of uniting with five molecules of acetic, nitric and benzoic acid; this indicates the presence of five hydroxyl groups. The presence of an aldehyde group is indicated by the reducing power of glucose and by its ability to combine with phenylhydrazine. Glucose is optically active, rotating the plane of polarized light to the right, the specific rotation, i.e., the number of degrees the plane of a polarized beam of light is rotated when passed through one decimeter of a solution containing 1 gm. of the substance per cc., being about 52.5°. Freshly dissolved glucose shows a greater specific rotation than this, the value eventually decreasing to 52.5°, or immediately upon heating

or treating with a drop of ammonia. This phenomenon is called muta-rotation.

When d. glucose is treated with methyl alcohol and dry hydrochloric acid, two methyl glucosides are formed, the alpha (long needles) with a rotation of +157°, and the beta (rectangular prisms) with a rotation of −33°. The alpha methyl glucoside separates on evaporating the mixture at temperatures below 98°C. On evaporation at temperatures above 98°C. the beta methyl glucoside separates.

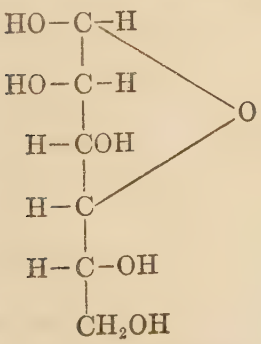


alpha methyl glucoside

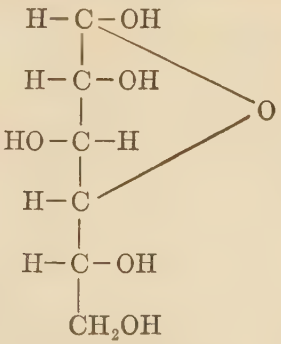


beta methyl glucoside

The formation of these two glucosides makes it necessary to assume the existence of two isomers of d. glucose which can be represented graphically by butylene-oxide formulas.



alpha d. glucose



beta d. glucose

The phenomenon of mutarotation is also explained by this assumption.

Detection and quantitative estimation of d. Glucose.— Most tests for glucose depend upon its reducing power. Alkaline silver solutions treated with glucose deposit a mirror of metallic silver. Alkaline copper solutions on heating with glucose form a precipitate of cuprous oxide. An alkaline bismuth solution gives a precipitate of metallic bismuth (Almén-Nylander test). Picric acid upon heating with glucose and sodium carbonate is reduced to form the intensely brown picramic acid. This last reaction has been successfully used by Lewis and Benedict for the quantitative estimation of blood sugar. It must be borne in mind that the reduction tests are not specific for d. glucose, but are given by all sugars containing free aldehyde or ketone groups.

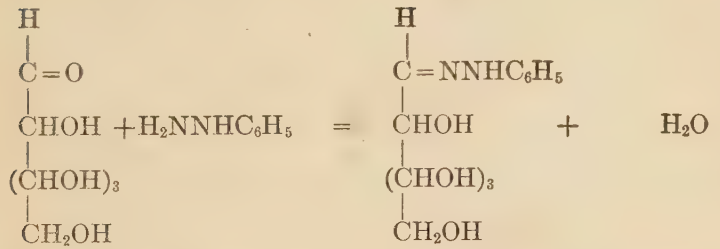
The test most frequently used for the detection of glucose in urine up to the present time has been Fehling's test. Fehling's solution consists of a freshly prepared mixture of a solution of copper sulphate and of a solution containing sodium hydroxide and Rochelle salt, the Rochelle salt (sodium potassium tartrate) serving to keep copper hydroxide from precipitating. The drawbacks of this reagent are that the strong alkali is likely to destroy some of the sugar and that a reduction of the copper may sometimes be brought about by the phenols, uric acid and creatinine, which are present in urine.

S. R. Benedict has devised a qualitative sugar reagent which is better suited for the detection of sugar in urine than is Fehling's solution. Benedict's reagent contains in one solution, copper sulphate, sodium carbonate as the alkaline constituent and sodium citrate as a solvent for the copper hydroxide. The solution keeps well. Eight drops of urine are added to five cc. of this reagent and the mixture is boiled for exactly two minutes.

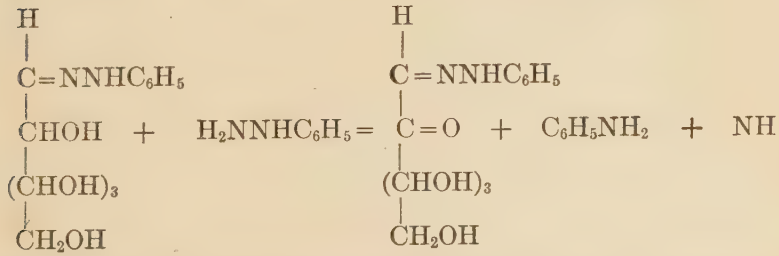
One of the most specific reagents for sugar in urine is a solution containing dinitrosalicylic acid, Rochelle salt, sodium bisulphite, sodium hydroxide and phenol. This reagent can be used directly to determine the small amount of sugar that is found in normal urine.

The Molisch test is given not only by glucose but by all carbohydrates and by all substances containing carbohydrate groups. This test is performed by treating the solution of carbohydrate with a few drops of an alcoholic solution of alpha naphthol. Strong sulphuric acid is then stratified in the bottom of the test tube. If carbohydrate is present, a purple ring develops.

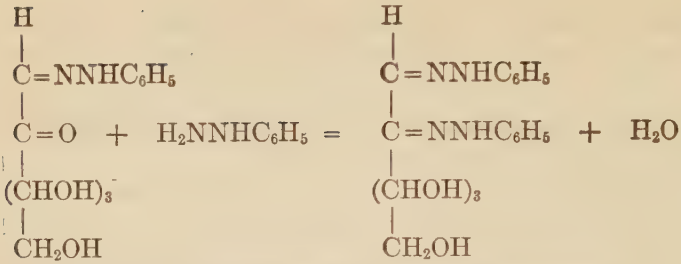
When glucose is treated with phenylhydrazine, the compound glucosehydrazone is formed. Upon heating in the presence of phenylhydrazine and a little acetic acid glucosazone is formed, and precipitates as yellow crystals. The reactions are as follows:



glucose phenylhydrazine glucose phenylhydrazone

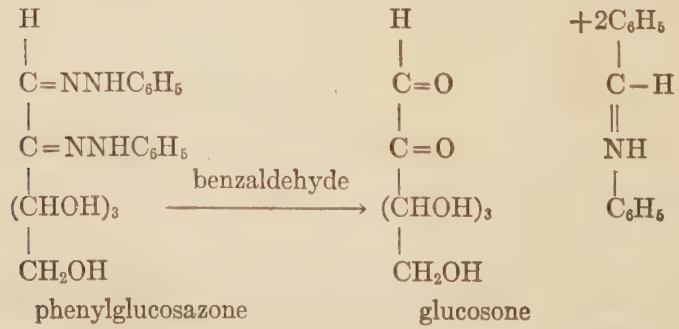


glucosephenyl- hydrazone phenylhydrazine glucosone phenylhydrazone aniline ammonia

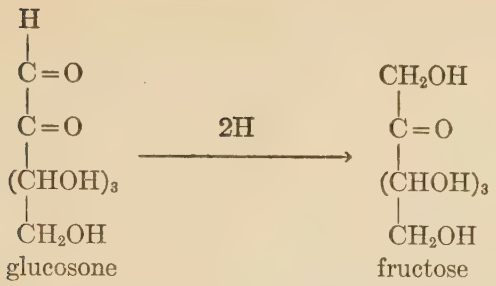


glucozone phenylhydrazine phenylglucosazone phenylhydrazone

The formation of osazones has been of great importance in the study of the sugars. Inasmuch as nearly all of the sugars can be converted into osazones, it is possible to use this reaction for the purpose of identifying sugars. This can be done by observing the habit of the osazone crystals, or by determining their melting point or their specific rotation. In the case of certain sugars the use of substituted phenylhydrazines is advisable, viz., methyl phenyl hydrazine. Osazone formation is of value as a means of purifying sugars when large amounts of impurities are present. After isolation, the osazone, upon treatment with hydrochloric acid or benzaldehyde, is converted into an ozone and this can be reduced to a ketose.



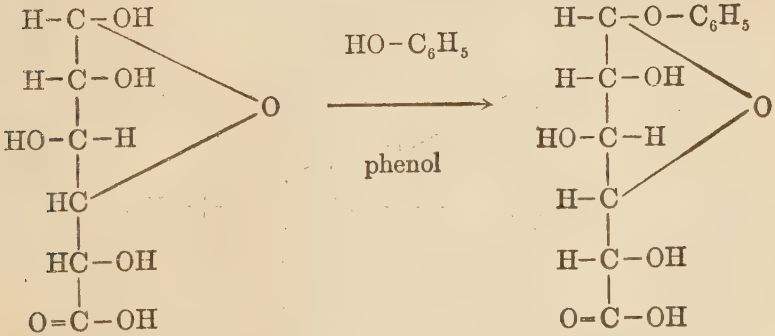
phenylglucosazone glucosone



glucosone fructose

Upon reduction d. glucose forms a hexahydric alcohol, sorbitol or sorbite, which is frequently found in plants.

Upon oxidation with bromine glucose is converted into gluconic acid, the aldehyde group being changed to a carboxyl. Boiling with dilute nitric acid oxidizes both ends of the glucose molecule with the formation of saccharic acid. By treatment with hydrogen peroxide the aldehyde group in glucose is not affected but the opposite end of the molecule is oxidized, giving rise to glycuronic acid. This substance is of especial importance to the biochemist as it is formed by the animal cell. (Compare Dakin's oxidation of butyric acid by hydrogen peroxide to substances which are formed in fat catabolism.) Glycuronic acid is used by the animal cell to detoxicate such poisonous substances as phenol, skatoxyl and indoxyl. In the putrefaction of pro-

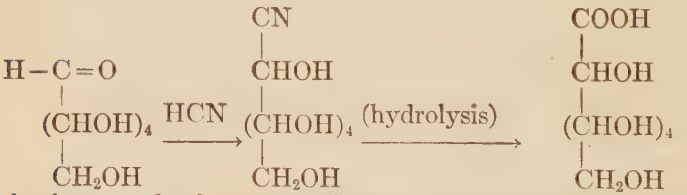


butylene oxide form of glycuronic acid phenyl glycuronate

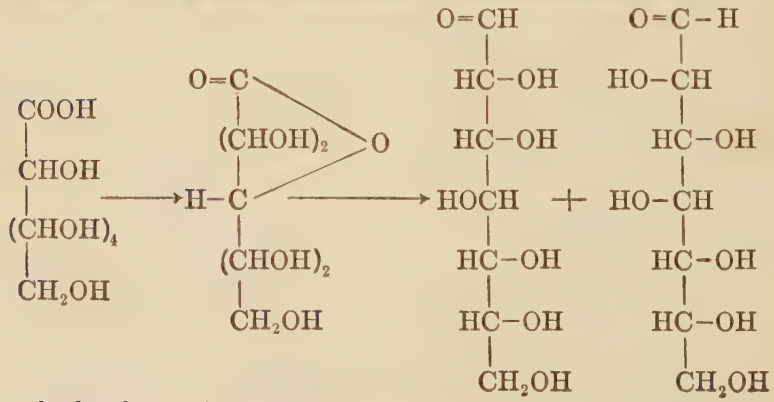
teins in the intestinal tract skatol, indol, and phenols are formed. Upon absorption skatol and indol are oxidized to skatoxyl and indoxyl. The substances are united with glycuronic acid with a splitting out of water. The glycuronates formed are then excreted in the urine. A great variety of substances, such as camphor, thymol, turpentine and chloral hydrate are converted into glycuronates after being fed to animals.

Conversion of a Lower to a Higher Sugar.—We can convert a sugar with six carbon atoms into one with seven carbon atoms in the following manner. Glucose is treated with hydrocyanic acid forming a cyanhydrine. This, upon hydrolysis, gives glucoheptonic acid which by heating forms the gamma lactone. Upon reduction this yields glucoheptose. By repeating these reactions, sugars with eight and nine carbon atoms have been built up.

Reactions for lengthening the chain of a carbohydrate (E. Fischer):



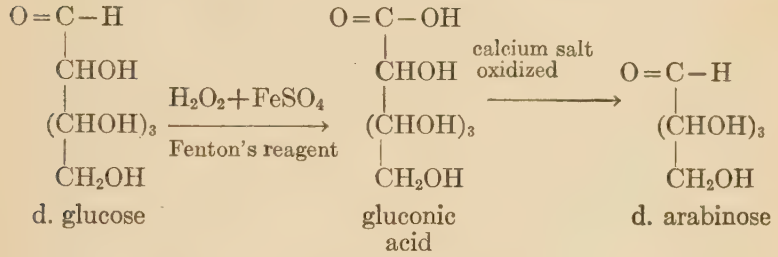
d. glucose d. glucose cyanhydrine d. glucoheptonic acid



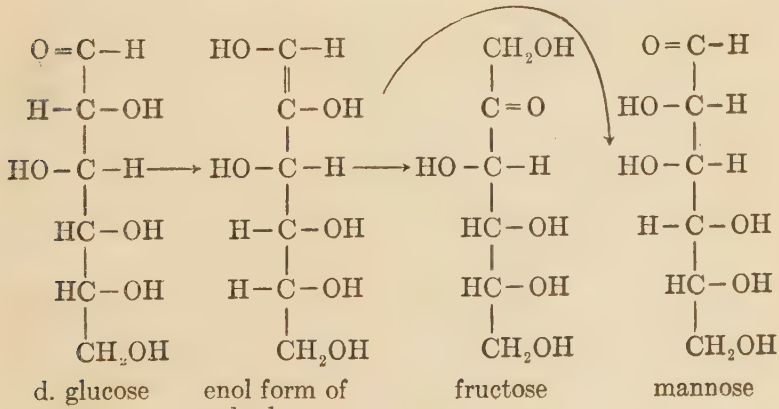
d. glucoheptonic acid — gamma lactone of d. glucoheptonic acid — d. glucoheptose + l. glucoheptose

The carbon atoms can be successively removed from a sugar in the following manner. Glucose is oxidized to gluconic acid by treatment with Fenton's reagent (H_2O_2 and $FeSO_4$). The calcium salt of gluconic acid upon further oxidation yields d. arabinose.

Reactions for shortening carbohydrate chain:



When glucose solutions are heated in the presence of alkali, a brown color develops and also the odor of caramel. This is Moore's test. A great many decomposition products, aldehyde resins among them, are formed in this reaction. In the presence of very dilute alkali glucose is slowly transformed into mannose and fructose through an intermediate enol form.



The enol form gives a purplish color with ferric chloride.

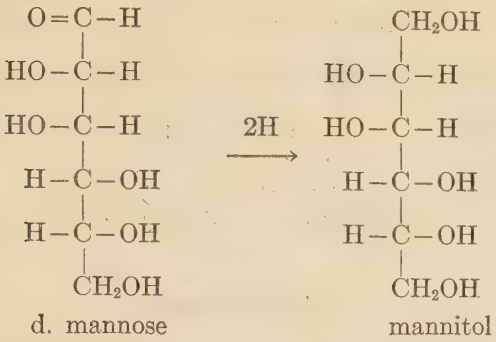
Glucosides.—It has already been mentioned that when glucose is treated with methyl alcohol in the presence of dry hydrochloric acid, two methyl glucosides are formed. A great many natural glucosides are found in plants. These can be looked upon as ether-like combinations of glucose and alcohols, acids, and aldehydes, often of the aromatic series. The glucosides are usually crystalline substances, soluble in water, bitter tasting, and often useful in medicines. While all true glucosides contain glucose, galactose and pentose are sometimes present also. Some examples of glucosides are:

amygdalin = 2 glucose + d. mandelo-nitrile.
sinigrin (black mustard) = glucose + allyl isothiocyanate + $KHSO_4$.
arbutin = glucose + hydroquinone.
phlorhizin = glucose + phloretin.
indican = glucose + indoxyl.
salicin = glucose + saligenin.
saporubrin (saponin) = sapogenin + several sugars.
ruberythric acid = 2 glucose + alizarin.

Glucosides are readily hydrolyzed by boiling with dilute acids, or by appropriate enzymes. The plant in which the glucoside occurs always contains enzymes capable of hydrolyzing this glucoside.

d. Mannose.—d. Mannose does not occur in nature in the free state, but is found in polysaccharides known as mannosans which are present in ivory nuts, coffee beans, date stones, and many other plant substances. These materials yield d. mannose upon hydrolysis. d. Mannose gives nearly all of the reactions of d. glucose. It forms the same ozazone upon treatment with phenylhydrazine, but is characterized by forming an insoluble alpha methyl hydrazone. Mannose

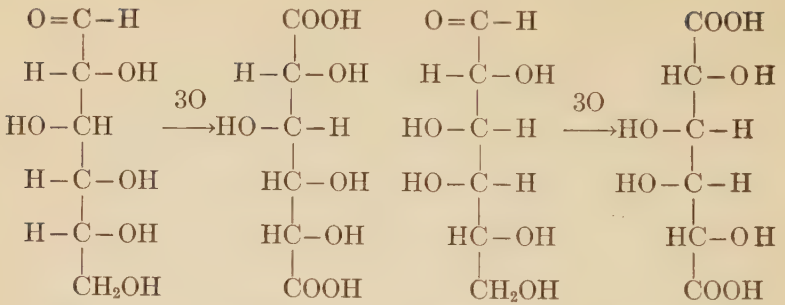
is readily fermented by yeast. Upon reduction mannose is converted into the hexatomic alcohol mannitol.



d. mannose mannitol

Mannitol is found in celery, olives, mushrooms, and syringa leaves. Considerable amounts are present in manna, an exudation from the manna ash (*Fraxinus ornus* of Sicily).

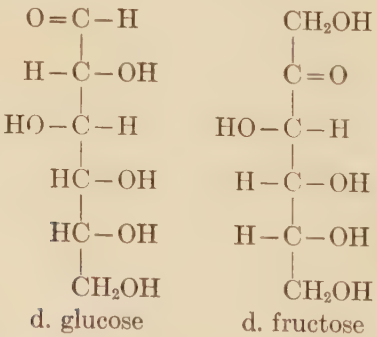
d. Galactose.—d. Galactose, like d. mannose, is found in nature only in combination with glucose or with other sugars. Milk sugar (lactose) contains one molecule of galactose and one of glucose. The cerebrosides, phrenosin and kerasin, contain galactose and can be classified as galactosides. As complex polysaccharides composed of galactose we have the galactans. Such occur in sea weeds (agar-agar and Irish moss). The pectins, which are present in plums, apples, peaches, and quinces, are similar to the galactans. d. Galactose undergoes alcoholic fermentation only with yeasts that have been especially trained for this purpose. It gives many of the tests shown by glucose, but can be differentiated by taste, by its ozazone and because of the fact that upon oxidation with dilute nitric acid, it forms the difficultly soluble mucic acid, a stereo-isomer of saccharic acid.



Keto-hexoses

Of the keto-hexoses only two of eight possible stereo-isomers are found in nature: d. fructose and d. sorbose.

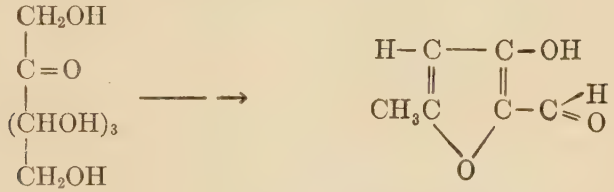
d. Fructose.—d. Fructose, otherwise known as levulose, or fruit sugar, is strongly levo-rotary, but has been named d. fructose by Emil Fischer because of the fact that it is closely related to d. glucose. Compare the constitution of the two substances:



Upon heating with phenylhydrazine d. fructose yields glucosazone. d. Fructose is present in the free state in honey and many ripe fruits. It rarely is found in the urine. The disaccharide cane sugar (sucrose) is composed of one molecule of d. fructose and one molecule of d. glucose. Inulin,

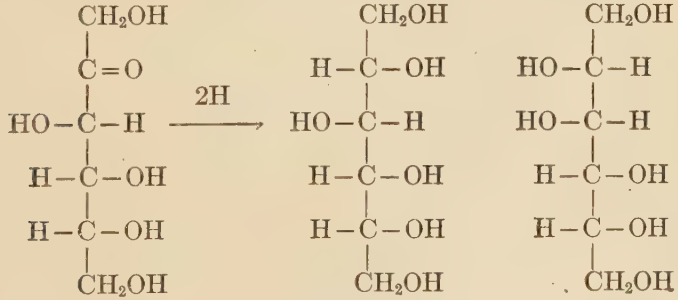
found in the Jerusalem artichoke and in dahlia roots, is a polysaccharide similar to starch but composed of fructose instead of glucose. One of the easiest methods for the preparation of d. fructose is the hydrolysis of inulin by heating with dilute acids.

d. Fructose readily undergoes alcoholic fermentation. It gives many of the tests shown by d. glucose. When heated with hydrochloric acid it yields 20 per cent of methyl hydroxy furfural. The aldohexoses yield only about 1 per cent of methyl hydroxy furfural (or a similar substance), under this treatment. On account of this fact fructose can be detected by heating with Seliwanoff's reagent, a solution of resorcin in hydrochloric acid.



d. fructose — methyl hydroxy furfural

Upon reduction d. fructose yields two hexahydric alcohols, d. sorbitol and d. mannitol.

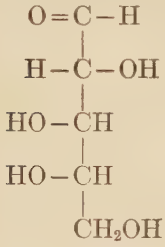


d. fructose — d. mannitol — d. sorbitol

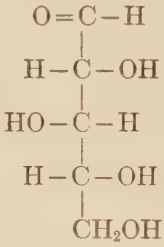
Upon oxidation the chain of carbon atoms is broken at the ketone group with the formation of various organic acids.

Pentoses

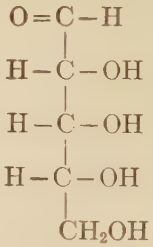
Of eight possible aldopentoses, three occur in nature: l. xylose, l. arabinose, and d. ribose.



l. arabinose (dextrorotatory)

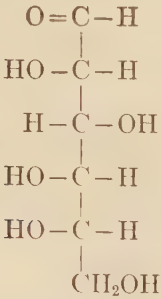


l. xylose (dextrorotatory)

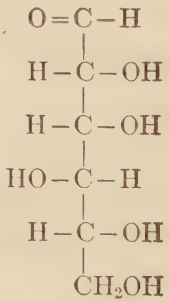


d. ribose

Compare l. arabinose with l. glucose and l. xylose with l. gulose.



l. glucose



l. gulose

These sugars are not usually found in the free state but are present chemically combined with certain proteins (nucleo-proteins) and as pentosans are widely distributed as gums and mucilages.

Pentoses are sometimes found in the urine, l. arabinose most frequently.

l. Arabinose is formed as one of the principal products from the acid hydrolysis of gum arabic and cherry gum. l. Arabinose, while dextro-rotatory, has been so named on account of its relationship to l. glucose.

l. Xylose is obtained by the acid hydrolysis of wood gum. It can be conveniently prepared from straw.

d. Ribose is said to be a constituent of some of the nucleoproteins.

The pentoses reduce the usual sugar reagents and form osazones with phenylhydrazine. While they are incapable of fermentation by ordinary yeasts, they are readily decomposed by bacteria and moulds. When pentoses are heated with strong hydrochloric acid they form about 90 per cent of furfural. This reaction is made the basis of tests for pentoses such as Tollen's and Bial's.

l. arabinose furfural

Quantitative Estimation of Pentoses.—For the quantitative estimation of the pentoses the sugar is treated with 10 per cent hydrochloric acid and the furfural is distilled off and treated with phloroglucin, with which it forms an insoluble phloroglucide. This is filtered off, dried, and weighed.

It should be remembered that while not all of the sugars described above are of importance as foods, they are, nevertheless, of great value to the bacteriologist for the identification of different kinds of bacteria, the method depending upon the ability of the culture in question to cause fermentation.

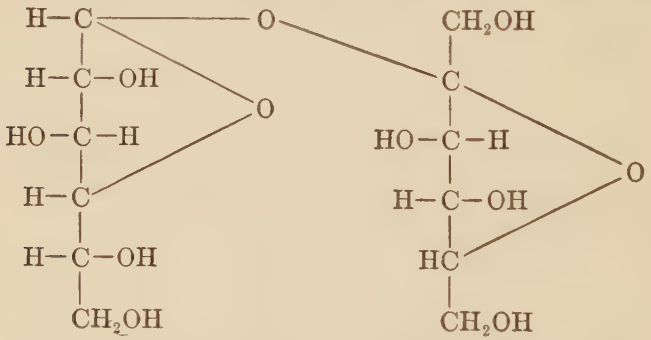
Disaccharides

The three important disaccharides are sucrose, maltose, and lactose. These sugars can be considered as ether-like combinations of two mono-saccharide molecules.

Sucrose.—Sucrose, saccharose, or cane sugar is composed of one molecule of d. glucose and one of d. fructose. Sucrose

is found in most ripe fruits and is often present also in the sap of the plant. The sugar cane may contain as much as 18 per cent sucrose, and the sugar beet still more. The pineapple contains 11 per cent and the strawberry 6 per cent. Commercial sources are sugar cane, sugar beet, maple tree, palm tree, and sorghum.

Cane sugar is not found in animals. The most probable structural formula represents sucrose as both a glucoside and a fructoside of the gamma oxide forms of these sugars.



sucrose (formula of E. Fischer.)

This structural formula explains the inertness of sucrose. As no aldehyde or ketone groups are present we can see why sucrose has no reducing action and why it will not react with phenylhydrazine until it is hydrolized.

While a solution of d. glucose is probably a mixture of three substances (the aldehyde form and the alpha and beta butylene oxide forms), there exists but one sucrose. This is probably the reason why sucrose crystallizes so readily. The commercial value of sucrose is partly due to its power to crystallize and to its inertness.

Sucrose is far more easily hydrolized than the other disaccharides. Heating with dilute organic acids is sufficient to cause this reaction. The enzyme invertase or sucrase which is present in ordinary yeasts, hydrolyzes sucrose very rap-

idly. For this reason yeast is able to hydrolyze sucrose and then ferment the monosaccharides which are formed.

Sucrose is strongly dextro-rotatory. The products of hydrolysis are slightly levo-rotatory because of the fact that d. fructose is more strongly levo-rotatory than d. glucose is dextro-rotatory. Therefore the hydrolysis of sucrose is termed inversion and the product is called invert sugar.

Maltose.—Maltose, or malt sugar, is found in the cell sap of leaves and is possibly present in small amounts in the animal body. It is formed as an end product of the hydrolysis of starch and glycogen by amylases (ptyalin, amylopsin), and is an intermediate product in the acid hydrolysis of these carbohydrates.

Maltose can be decomposed by boiling with acids to form two molecules of d. glucose. This hydrolysis is also effected by the enzyme maltase. As maltase is present in ordinary yeast, yeast is capable of hydrolysing maltose and later fermenting the glucose formed.

Maltose is a reducing sugar and forms an osazone. These facts indicate that an aldehyde group is present. Maltose can be distinguished from d. glucose by its inability to reduce Barfoed's reagent (copper acetate and acetic acid), except upon prolonged heating.

Iso-maltose is a disaccharide formed by the action of hydrochloric acid or the enzyme maltase upon 40 per cent glucose solution. Small amounts of this sugar are produced by reversion during the digestion of starch.

Lactose.—Lactose, or milk sugar, is found only in milk, although in cases of milk suppression and sometimes during pregnancy it may be present in the urine. Upon hydrolysis by acids and by the enzyme lactase, lactose is broken down into glucose and galactose. Ordinary yeasts do not contain the enzyme lactase and are consequently without action upon lactose. In Asia use is made of certain yeasts that do

contain lactase to ferment milk with the production of such beverages as kephir and koumyss.

Lactose crystallizes well and has a chalky taste with a trace of sweetness. Solutions of lactose readily undergo lactic and butyric acid fermentations. Lactose is a reducing sugar, forms an osazone and gives the mucic acid test.

Raffinose.—Raffinose is a trisaccharide, being composed of glucose, galactose and fructose. It is present in the sugar beet, in sugar cane after a frost, in barley, cottonseed and manna. Upon hydrolysis by the enzyme sucrase, fructose is split from the raffinose molecule, leaving the glucose and galactose combined as the disaccharide *melibiose*. Emulsin splits raffinose into sucrose and galactose. An enzyme attacking melibiose is present in bottom yeasts.

Theory of Synthesis of Carbohydrates

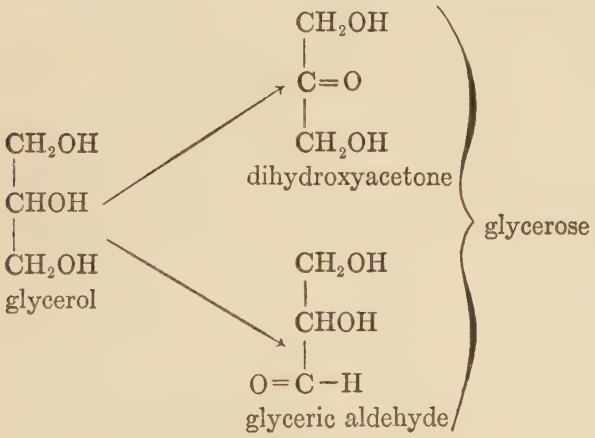
In 1870 Baeyer suggested that in the synthetic formation of sugar by plants under the influence of sunlight, chlorophyll is able to decompose carbon dioxide and water with the formation of formaldehyde, the formaldehyde being almost instantly polymerized into one of the sugars. Formaldehyde has been found in traces after exposing the green leaves of plants to the sunlight. It is interesting to note that a possible intermediate product in the formation of formaldehyde, namely, carbon monoxide, has been found to be formed by a species of kelp.

Laboratory Syntheses.—Turning to attempts of the chemist to imitate the plant, we find that formaldehyde can be formed from carbon dioxide and water in the presence of metallic magnesium by the aid of sunlight. Baly has shown that ultra-violet light of a certain wave-length is capable of producing aldohexose sugars as well as formaldehyde from carbonic acid and he suggests that the formaldehyde is not an intermediate product, but that it is

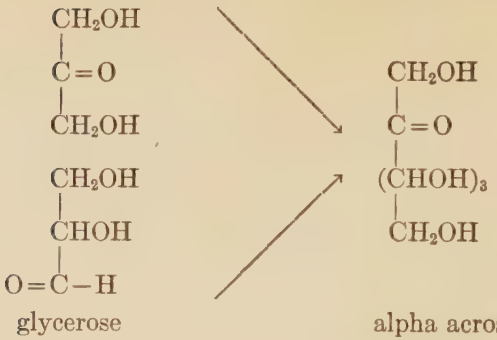
formed rather as a degradation product of the action of the ultra-violet light on the sugars.

When formaldehyde is treated with lime water, it polymerizes and forms a mixture of unfermentable sugars known as formose (empirical formula $C_6H_{12}O_6$). When polymerized by magnesia, formaldehyde is converted into a mixture of sugars which is known as methose (empirical formula $C_6H_{12}O_6$). In this case a part of the sugar can be fermented by yeast.

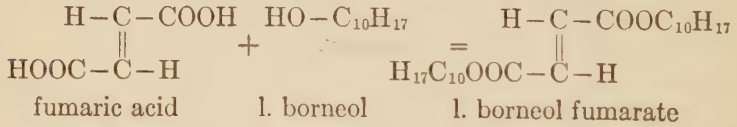
If one oxidizes glycerol with sodium hypobromite, the principal product is dihydroxy-acetone. Small amounts of glyceric-aldehyde are also produced. When this mixture, which is known as glycerose, is allowed to stand in contact with dilute sodium hydroxide solution, alpha and beta acrose are formed. Alpha acrose has been shown to be

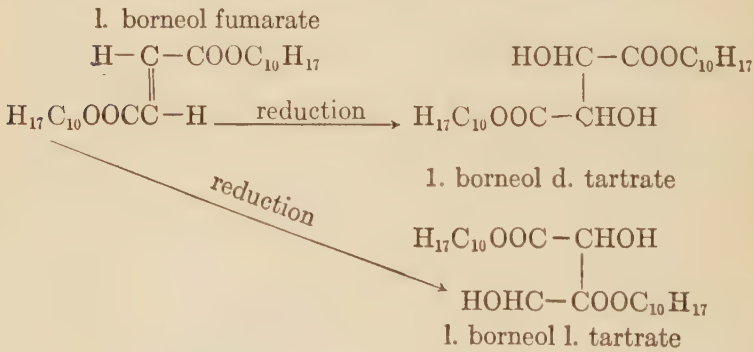


d. l. fructose. Upon submitting this to the action of yeast, the d. fructose is fermented, leaving the l. fructose. Alpha acrose is also present in the methose referred to above. It can also be prepared by treating acrolein dibromide with barium hydroxide.

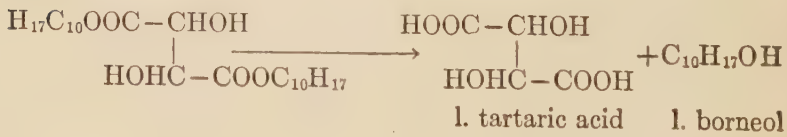


It should be noted that while synthesis by the living cell is often asymmetric, synthesis in the test tube will be symmetric, the chances being equally good for the formation of either enantiomorph. Asymmetric compounds have been synthesized, however, by processes of the following sort:





The l. tartrate predominates:



Polysaccharides

The polysaccharides are unreactive and of high molecular weight. They are insoluble in water, form colloidal solutions and for the most part will not diffuse through parchment or animal membranes. The constitutions of the polysaccharides are almost entirely unknown. Indeed this field is probably the most difficult one in organic chemistry. The most that we can say about the polysaccharides is that they are anhydrides of the sugars, and that upon hydrolysis by acids or enzymes, they are broken down first into intermediate products and finally into the monosaccharides.

Cellulose.—Cellulose, the predominating organic compound of plants, can be looked upon as a supporting material. It is characterized by its inertness and by its insolubility. Cellulose is not hydrolyzed by boiling with dilute acids, but if dissolved in concentrated sulphuric acid and then diluted, heating will effect hydrolysis, with the intermediate production of the disaccharide cellose and the ultimate formation of glucose. Cellulose forms a number of nitrates upon treatment with a mixture of strong nitric and sulphuric acids. The most important of these are the tetranitrate (pyroxylin) and the hexa-nitrate (guncotton). Cellulose can be dissolved in ammoniacal copper hydroxide solution and in various mixtures containing hydrochloric acid and metallic chlorides. Artificial silk is made by dissolving cotton in this manner and afterwards precipitating it as a thread.

Wood is composed of cellulose, hemicellulose, lignin and resin. Spruce wood contains 53 per cent of cellulose.

Starch.—Starch occurs in seeds, nuts, roots, tubers and fleshy leaves as a reserve food. The starch granules, which vary in shape and size, depending upon the species of plant, can be considered composed of starch granulose and starch pectin, surrounded by a covering of starch cellulose. On

heating with water to about 80° C. the envelope is ruptured, and the granulose and pectin form a colloidal solution. Starch granulose has recently been crystallized.

Starch is precipitated by tannic acid and by adding alcohol (to a concentration of 50 per cent). It can be salted out by the addition of ammonium sulphate. By the action of amylases (ptyalin, amylopsin, malt diastase, etc.), starch is hydrolyzed to maltose; by heating with acids, starch is hydrolized to glucose.

Starch is comparatively unreactive. It is best detected by the blue color which it gives with iodine. To estimate starch quantitatively, one converts it by hydrolysis to glucose and determines the glucose by one of the reduction methods.

Dextrin.—Starch is converted into dextrin by heating in an oven until it begins to brown. If the starch is first moistened with a little dilute nitric or hydrochloric acid, the dextrin formation takes place at a lower temperature. Dextrin is also formed as an intermediate product of hydrolysis by enzymes. There are said to be at least four different dextrins: amylo-dextrin (colored blue by iodine), erythro-dextrin (colored red by iodine), and achroö- and malto-dextrins (giving no color with iodine).

Lichenin.—Lichenin from Iceland moss is a polysaccharide closely related to starch. It forms glucose upon hydrolysis. *Inulin* is a variety of starch built up from fructose molecules. Neither lichenin nor inulin is colored by the addition of iodine.

Glycogen.—Glycogen or animal starch is found in most animal cells, but especially in the liver (12-16 per cent). Smaller amounts are present in the muscles. Considerable amounts of glycogen are found in oysters, scallops, mushrooms, bacteria and yeast cells. After the death of an animal the glycogen is rapidly converted to maltose, glucose and finally to lactic acid. The constitution of glycogen is

probably similar to that of starch. Its specific rotation is nearly the same (196°). With iodine it gives a slight reddish brown color. Glycogen forms a colloidal opalescent solution with water, from which the glycogen can be precipitated by adding alcohol to a concentration of 60 per cent. Glycogen is hydrolyzed by amylases with the formation of maltose. With acids it is hydrolyzed to glucose.

Carbohydrate Digestion

The digestion of carbohydrates can be considered as a hydrolysis of the polysaccharides into disaccharides, and a further hydrolysis of the disaccharides to monosaccharides.

In the mouth the amylase ptyalin in the saliva starts the hydrolysis of starch, converting the starch into dextrins and finally into maltose. Small amounts of glucose and isomaltose are said to be formed in this process. Ptyalin acts readily upon boiled starch but very slowly upon raw starch. Ptyalin is said to be absent from the saliva of carnivorous animals.

The optimum reaction for ptyalin is a very faint acidity and as strong acids quickly inactivate the enzyme, it can be seen that starch digestion will not continue in the stomach contents after these have been mixed with the acid gastric juice. The salivary digestion of starch probably continues for some time in the upper, or fundic, portion of the stomach, as little acid is secreted in this region.

In the small intestine the digestion of starch to maltose is brought about by the amylase from the pancreatic juice, amylopsin. In the intestinal juice or succus entericus occur three enzymes: sucrase, which splits sucrose; maltase, which splits maltose; and lactase, which splits lactose. The optimum reaction for all of these intestinal enzymes is a faint acidity, such as obtains in the middle and upper portions of the small intestine. The end products of carbohydrate digestion on a normal diet are for the most part

glucose with smaller amounts of fructose, galactose and pentoses. Micro-organisms present in the digestive tract normally bring about to a small degree the fermentation of carbohydrate materials, forming hydrogen and marsh gas from cellulose; the fermentation of sugars yields carbon dioxide and alcohol, and lactic and butyric acids.

When excessive amounts of sugar have been ingested, and when because of constipation the process of digestion is interfered with, a considerable amount of fermentation may take place in the digestive tract. As the products of fermentation are harmless, a moderate amount of fermentation is considered advantageous because fermentative bacteria are capable of preventing processes of putrefaction. In putrefaction poisonous products are formed. See page 116.

Carbohydrate Absorption

The monosaccharides produced by carbohydrate digestion are absorbed through the intestinal wall and pass by way of the capillaries into the portal vein. Normally the absorption of carbohydrate is about 95 per cent, but after ligation of the pancreatic duct it falls to about 50 per cent.

Cori has found that in rats the hexose sugars are absorbed from the intestine at a rate which is constant for each sugar and which is independent of the concentration of the sugar in the intestine. He has used the term *"absorption coefficient"* to denote the amount of sugar absorbed per

Type of sugar	*absorption coefficient*
d. galactose	0.196
d. glucose	0.178
d. fructose	0.077
d. mannose	0.034
l. xylose	0.028
l. arabinose	0.016

Inasmuch as all of these sugars are soluble in water and readily diffusible the marked differences in their rates of absorption are evidently due to some unknown property or action of the cells of the intestinal epithelium.

unit of body weight per hour. The absorption coefficients for some sugars are given below. It will be noticed that the rate of absorption varies widely, depending upon the sugar.

The portal vein terminates in the liver. This organ takes out the excess of sugar present in the blood; for while the amount of sugar in the portal blood during absorption may amount to 1 per cent the quantity in the systemic circulation is about 0.1 per cent. The various hexoses retained by the liver are stored as glycogen. Glucose and fructose are readily transformed into glycogen while galactose forms glycogen less easily. The pentoses are probably not converted into glycogen. It seems evident that the liver must possess the ability of converting fructose, galactose, and possibly mannose into glucose, since but one kind of glycogen has ever been found, namely, the anhydride of glucose.

Carbohydrate Metabolism

The quantity of glucose present in the blood is about 0.1 per cent. This value is kept very constant. As fast as glucose is used up by the tissues, glycogen stored in the liver, muscles, and other organs, is mobilized as glucose. It seems probable that all cells, both plant and animal, can either burn or ferment glucose.

Respiratory Quotient.—We can tell whether an animal is burning glucose by finding the respiratory quotient. The respiratory quotient is

$$\frac{CO_2 \text{ vols. expired}}{O_2 \text{ vols. absorbed}}$$

If an animal were burning pure carbon, every volume of oxygen absorbed would be converted into one volume of carbon dioxide, since the reaction is: $C+O_2=CO_2$. The carbohydrates, with unimportant exceptions, can be given the formula $C_x(H_2O)_y$. From this we see that there is

enough oxygen present in each of these carbohydrates to convert all of the hydrogen into water. When the animal burns carbohydrate, no more oxygen will be needed than would be required to burn pure carbon. Hence for every volume of oxygen absorbed, one volume of carbon dioxide will be produced in the physiological oxidation of carbohydrates. The respiratory quotient will be 1. We find that an animal which has recently been fed carbohydrates has a respiratory quotient of 1, or nearly 1.

A starving animal is burning fat for the most part, and here we find that the respiratory quotient is approximately 0.7. Consideration of the formula for olein, $C_{57}H_{104}O_6$, shows that with a compound of this sort, after deducting enough hydrogen to form water with all of the combined oxygen, a considerable amount of hydrogen still remains. In this case, more oxygen will be needed for the combustion. So the respiratory quotient will be less than 1. The reaction and computation involved in obtaining the respiratory quotient for olein is given below:

$C_{57}H_{104}O_6$, or olein	57 C	+ 57 O_2	=	57 CO_2
	6 H_2O			6 H_2O
	46 H_2	+ 23 O_2	=	46 H_2O
		80 O_2		57 CO_2
		oxygen absorbed		carbon dioxide produced

$$\frac{57\ CO_2 \text{ vols. produced}}{80\ O_2 \text{ vols. absorbed}} = 0.71 \text{ respiratory quotient}$$

An animal living on protein has a respiratory quotient of about 0.8. The respiratory quotient calculated for the combustion of alcohol is 0.667 (calculate this yourself).

Oxidation of Glucose.—Each gram of glucose yields 4.1 large calories upon oxidation. The reactions involved in this oxidation are not known and the enzymes involved have never been satisfactorily demonstrated in vitro. It

seems likely that insulin (which will be discussed later) is responsible for the production in the cells, of some more reactive form of glucose. This product is probably next converted into glucose phosphate, a substance known to be produced during the fermentation of glucose by yeast. The next stage in the catabolism of glucose is very debatable. Glyceric aldehyde, dihydroxy acetone, methyl glyoxal and glycollic aldehyde have all been proposed as intermediate products. These substances are all capable of giving rise to glucose when fed to a diabetic animal.

Lactic acid is known to be formed by the living cells from glucose (or rather from glucose phosphate) whenever there is a lack of oxygen. A. V. Hill has found that during muscular contraction there is an initial rapid liberation of a small amount of heat (and a production of lactic acid if oxygen is absent). If oxygen is present there is a later liberation of a much larger amount of heat and a disappearance of the lactic acid. It is claimed that lactic acid is not oxidized but that it is converted into glycogen in the process of recovery after the muscular contraction (Myerhof). After death the glucose present in animal tissues is rapidly converted to lactic acid. This process also takes place in samples of blood and is called "glycolysis."

Glycosuria

Glycosuria can be defined as a condition where glucose is present in the urine. As normal urine contains no glucose, glycosuria can be regarded as an abnormal state. Although normal urine contains no glucose, it does contain small quantities of sugar or sugars which have a reducing action and which are in part destroyed by incubation with yeast. The nature of these sugars is unknown. Folin believes them to be fragments produced in the carbohydrate metabolism, or formed in the cooking of food. Benedict has proposed the term "glycuresis" for the process of elimi-

nating sugar in normal urine. Benedict and Osterberg state that the normal elimination of sugar does not exceed 1.5 gm. in 24 hours. The elimination is increased after meals.

Detection and Estimation of Sugar in Urine.—The detection of small amounts of glucose in urine is made difficult not only on account of the reducing action of such constituents of the urine as phenols, uric acid, creatinin and glycuronic acid, but also on account of the presence of the unknown reducing sugars. Benedict's qualitative sugar reagent has been so devised that when used according to directions no precipitate is given unless the urine contains an abnormal amount of reducing substance. The sugar of normal urine can be determined by the picric acid-acetone method of Benedict and Osterberg, after first removing interfering substances by shaking the urine with a specially prepared animal charcoal. Folin and Berglund have determined the sugar of normal urine by means of the Folin-Wu blood sugar reagents, after shaking the urine with Lloyd's reagent (a purified clay) and sulphuric acid. The Sumner method for determining sugar in normal urine depends upon the use of a reagent containing dinitrosalicylic acid, sodium hydroxide, Rochelle salt, phenol and sodium sulphite. The dinitrosalicylic acid in alkaline solution is reduced by heating with the sugar, to an intensely colored brown compound which is probably nitro-amino salicylic acid. The Rochelle salt is added to keep oxygen from dissolving in the reagent and to prevent the precipitation of calcium. The phenol has been added to the reagent because the phenols in urine have an intensifying effect upon the reduction of dinitrosalicylic acid by sugar. When the reagent contains an excess of phenol the standard is affected just as much as the unknown. The sodium sulphite in the reagent keeps the color of the reduction compound from fading. With the dinitrosalicylic test, concentrated urine that is found to contain more than 0.18

per cent of sugar or dilute urine that is found to contain more than 0.12 per cent of sugar can be considered to be abnormal.

Renal Glycosuria.—In certain instances persons who are apparently in good health are found to have a continual glycosuria. As much as 5 per cent of glucose may be present in the urine. This glycosuria is more or less independent of the diet and is believed to be caused by an abnormal permeability of the kidneys. Subjects with renal diabetes, as this condition is called, have been shown to oxidize sugar as readily as normal persons.

Alimentary Glycosuria.—When a person is fed more than 200 grams of glucose, the power of the liver to store sugar as glycogen is exceeded. The blood sugar increases so much above its normal amount that the excess passes into the urine. The renal threshold is somewhere between 0.14 and 0.18 per cent. In this case we are dealing with alimentary glycosuria, and we say that the assimilation limit for glucose has been exceeded. The assimilation limit for sucrose is about 200 grams; for lactose from 60-120 grams; for pentoses from 30-60 grams. The assimilation limit for starch is practically infinite, the reason being that starch requires time to be digested and consequently will be absorbed slowly. The assimilation limit for different sugars varies with individuals. Where there is a tendency towards diabetes the sugar tolerance is low.

Emotional Glycosuria.—Glycosuria is sometimes caused by fright or worry. This does not happen when the liver is free from glycogen. This is termed emotional glycosuria. Injecting solutions of adrenalin will invariably bring on glycosuria even when there is no glycogen in the liver. Stimulation of the splanchnic nerve or the central end of the vagus and piqûre, or sugar puncture, in the floor of the fourth ventricle between the eighth and tenth cranial nerves, will cause glycosuria if the liver contains glycogen.

Giving such drugs as caffeine or strychnine produces glycosuria; so, too, does asphyxiation.

Hyperglycemia

It appears that all of the glycosurias above mentioned are connected with an excess of sugar in the blood, or a hyperglycemia.

Renal Threshold.—Although small amounts of sugar (0.01–0.18 per cent) are always present in the urine, it is extremely improbable that any of this sugar is d. glucose. The kidneys do not allow d. glucose to pass into the urine until the percentage in the blood has reached a value of somewhere between 0.14 and 0.18 per cent. This value is known as the "renal threshold."

Such substances as creatinin and urea have no renal threshold.

Hypoglycemia

Administration of the glucoside phloridzin causes glycosuria. In this case the glycosuria is probably due to an increased permeability of the kidneys as well as to the inability of the cells to burn glucose. In phloridzin poisoning we should expect to find a decreased amount of sugar in the blood, provided the leakage through the kidneys is large. Where there exists a diminished amount of blood sugar the condition is called hypoglycemia. Administration of uranium salts also produces a glycosuria on account of kidney damage. A similar sort of glycosuria is produced by intravenous injection of very large quantities of 1 per cent sodium chloride solution. This is stopped by the injection of calcium chloride solution.

Diabetic Glycosuria

One other sort of glycosuria remains to be discussed. This is the glycosuria of diabetes mellitus. Diabetic glycosuria is always found to be the immediate result of a hyper-

glycemia, and not to be due to an increased permeability of the kidneys. Indeed, in long standing cases of diabetes the sugar threshold of the kidneys is always found to be raised, often above 0.2 per cent. This possibility indicates that the quantitative estimation of blood sugar is more to be relied upon than is the quantitative determination of sugar in the urine.

We can propose three explanations for diabetic hyperglycemia:

1. The animal has lost its ability to oxidize glucose.
2. The animal is incapable of storing glycogen.
3. Excessive amounts of glucose are synthesized from protein or fat.

Carbohydrate Catabolism in Diabetes

Examination of respiratory quotients of diabetic animals shows that little or no carbohydrate is being burned. Instead of a value of 1 we find values of 0.7 to 0.8. We should expect that if carbohydrates could be burned, vigorous exercise would use up enough glucose to stop the glycosuria. But this is not the case. It has been shown that the surviving diabetic heart perfused with diabetic blood uses less glucose than the normal heart when perfused with normal blood.

Glycogen Storage in Diabetes

That the diabetic animal is incapable of storing glycogen in the liver is shown by autopsies after death from diabetes. Some glycogen is found in the muscles, but none in present in the liver. However, this lack of glycogen in the liver is probably due to the fact that glycogen is broken down as fast as it is formed. The sugar-hungry cells are constantly calling for sugar.

In a normal animal, when there is a plentiful supply of carbohydrate, some of this carbohydrate is transformed into

fat. The formation of fat from carbohydrate either does not occur in diabetes, or else the fat that is formed is used up at such a rapid rate that none is deposited in the tissues.

Synthesis of Glucose in Diabetes

In advanced cases of diabetes more sugar is present in the urine than can be accounted for by the carbohydrate of the food. Some of this sugar must have been synthesized from protein, or from fat. It has been found that feeding protein causes an increase in the amount of sugar excreted, and Stiles and Lusk have shown that this sugar is formed by the animal from certain amino acids which are present in the protein molecule. It should be understood that the normal animal, as well as the diabetic animal, synthesizes sugar from protein. It appears very improbable that sugar is ever formed from fat if we except the glycerol of the fat.

The D/N Ratio.—From consideration of the elementary composition of proteins, we can calculate that 80 per cent of the protein molecule might be transformed into sugar. Actually we never find more than 61 grams of glucose produced from 100 grams of protein. The urine of a totally diabetic animal will contain both the nitrogen and the glucose produced in protein catabolism. It is customary to express glucose production from protein in terms of what is known as the D/N ratio, that is, $\frac{\textit{urinary dextrose (glucose)}}{\textit{urinary nitrogen}}$. The D/N value in phloridzin diabetes eventually reaches 3.6, indicating the production of 61.2 grams of glucose from every 100 grams of protein. (1 gm. of nitrogen is assumed to represent 6.25 gms. of protein, since 17 per cent of protein is nitrogen.) In experimental pancreatic diabetes the D/N ratio has a value of 2.8. The production of a greater quantity of glucose from protein in phloridzin diabetes than in pancreatic diabetes has never been explained. It may be due to the fact that a synthesis of fat occurs in pancreatic

diabetes and that some sugar is used in this manner. In phloridzin diabetes the kidneys are permeable and the blood sugar is low. This hypoglycemia might be expected to prevent fat synthesis, as fat is believed to be synthesized from carbohydrate in the normal animal only when the body has more sugar than it can store as glycogen.

The D/N ratio is of value as an indication of the severity of the diabetes. In the normal animal there is no sugar in the urine and the ratio is zero; in mild cases of diabetes the ratio is small; but as the severity of the disease increases less sugar is oxidized and more sugar appears in the urine. When the ratio in pancreatic diabetes or in diabetes mellitus has risen to 2.8 it has gone as high as it will go and indicates that the subject is totally diabetic, that is that no sugar at all is being oxidized. Accordingly this is called the fatal ratio. Ratios higher than 2.8 have been reported in diabetes mellitus, but these can be ascribed to the surreptitious eating of carbohydrate.

Role of the Pancreas in Carbohydrate Catabolism

The main point to explain in diabetes is the lack of oxidation of glucose. Some light has been thrown on this point by the work of von Mering and Minkowski. These men found that if they removed the pancreas of a dog, severe diabetes developed, many of the symptoms of diabetes mellitus being present. Removal of only 80 per cent of the pancreas did not result in the appearance of pancreatic diabetes as long as the remainder of the pancreas suffered no degeneration. Complete removal of the pancreas was found not to result in diabetes, provided a small portion of the pancreas was grafted into the spleen, or the abdominal wall. In diabetes mellitus lesions (hyaline degenerations) have sometimes been found in the pancreas, especially in that portion designated "the isles of Langerhans." It is evident that the pancreas plays some vital rôle in the carbo-

hydrate metabolism. It produces an internal secretion which is necessary for the oxidation of glucose. The external secretion of the pancreas (pancreatic juice) has nothing to do with the oxidation and storage of sugar.

Besides the fact that the pancreas is concerned with carbohydrate oxidation, we must also note that another function of the pancreas is to promote the storage of glycogen in the liver. In other words, the pancreas increases the sugar tolerance. Removal of the pancreas decreases the sugar tolerance. We can define sugar tolerance as the ability of the animal to keep the level of blood sugar from rising to any considerable degree. If a normal person is fed 100 gm. of glucose on an empty stomach, the blood sugar will rise from 100 to 130 or 150 mg. per 100 cc. of blood and will soon return to normal. In the diabetic person the blood sugar may increase to 250 or 300 mg. and will only slowly return towards normal.

Insulin.—Search for the pancreatic hormone proved unavailing for thirty years after the experiment of von Mering. In 1922 Banting and Best, working in the laboratory of J. J. R. McCleod, discovered the pancreatic hormone, which they named "insulin," a name which had been proposed by Shaefer many years before.

Banting and Best started with the idea that previous investigators had failed to prepare active pancreatic extracts owing to the simultaneous extraction from the pancreas of trypsin, which had a destructive action upon the hormone as well as a toxic effect upon the animal to whom the extracts were administered. They ligated the pancreatic ducts in dogs in order to cause the acinar tissue containing the trypsinogen to degenerate. After eight weeks the dogs were killed, and while the acinar cells were found to have degenerated, those of the isles of Langerhans had not. Upon making extracts of such material and injecting them into depancreatized diabetic dogs, the high blood sugar was

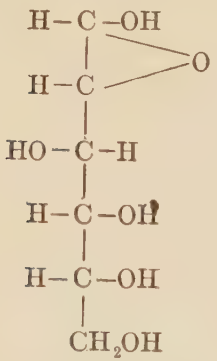
observed to fall, the urine became sugar-free, and for a short time the diabetic dogs were relieved from all the symptoms of the disease. Collip, who was invited to join the work, aided in the preparation of active extracts of insulin from undegenerated pancreases through the use of acidified alcohol, as this solvent in proper dilution dissolves the insulin but precipitates the trypsin together with the proteins.

Insulin has been found to be colloidal and although not a protein is possibly a polypeptide. Abel reports having obtained it in crystalline form. In acid medium it withstands boiling for a short time. It is inactivated by peptic and tryptic digestion and therefore cannot be given by mouth. It is administered by subcutaneous injection, usually three times a day, after meals. Murlin and his collaborators have succeeded in obtaining some absorption of insulin from the intestinal tract by feeding it enclosed in capsules containing citric acid. The capsules liberated the insulin in the intestine and the citric acid protected it from tryptic and ereptic digestion.

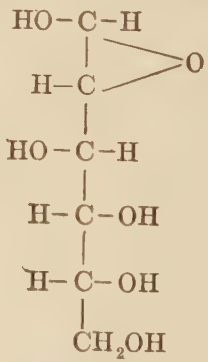
The dose of insulin that is injected must be regulated very carefully as an over-dose will bring about very alarming symptoms and may cause death. When an animal is given an over-dose convulsions come on and the blood sugar falls to a very low level. Cori has shown by ingenious analytical methods that less glycogen is formed in the liver and more sugar is oxidized, and that while insulin has depressed glycogen formation by the liver, glycogen formation by the muscles is not influenced. If prompt intravenous injections of glucose are given to an animal suffering from an over-dose of insulin relief is quickly obtained. In the diabetic animal the action of insulin is to cause oxidation of the blood sugar by the tissues and storage of glycogen in the liver. The mechanism by which this is accomplished has not been explained. It has been suggested that in the presence of insulin the alpha and beta forms of d. glucose

are changed to a form which is more readily converted into glycogen and which is easily oxidized by the cells.

Experimental evidence both for and against this theory is not lacking. Hewitt and Pryde injected glucose solutions into the intestines of rabbits. After removal of the solutions of glucose the specific rotation was found to have greatly diminished. Upon keeping the solutions *in vitro* the rotation eventually increased to the normal value. Winter and Smith made observations on the reducing and rotatory power of the sugar of normal blood and have found that for the amount of sugar calculated to be present by reduction, the rotation was considerably less than the specific value for glucose. When diabetic blood was examined the rotatory power was found to be normal. Winter and Smith believe that the glucose of normal blood is some form which is more reactive than ordinary glucose. They suggest that this may be gamma, or ethylene oxide glucose.



alpha ethylene d. glucose



beta ethylene d. glucose

Lundsgaard and Holbϕll have stated that when glucose is incubated with insulin and fresh muscle tissue at 37°, the reducing power is not affected, while the specific rotation falls sometimes to as little as 14°. They believe that the action of insulin is to convert glucose to a more reactive

form which is readily oxidized by the tissues. They have found that normal blood contains this more reactive form of glucose, which they call "new sugar," but that diabetic blood contains ordinary alpha and beta d. glucose.

As the results announced by these workers have been denied by Visscher, Denis, Barbour, Paul, Beard and Jersey, it is not yet time to accept them unreservedly.

While insulin has a very remarkable effect upon the diabetic animal, and will even restore a person in diabetic coma to consciousness within a few minutes, its effects are not lasting and it must be injected several times a day in severe cases of diabetes. It cannot cure diabetes, but if used in conjunction with the dietetic treatment it will undoubtedly be of the utmost use in helping the diabetic to recover from a severe condition and to progress to a condition where dieting alone is sufficient to maintain a satisfactory carbohydrate metabolism.

Influence of Other Ductless Glands.—Besides the pancreas, other ductless glands in the body have been found to have an influence on the storage of glycogen by the liver. Thus, while the parathyroids act similarly to the pancreas, the thyroid, the adrenals and the posterior lobe of the pituitary body have the opposite effect. In Basedow's disease, or hyperthyroidism, the sugar tolerance is lowered; in myxedema, a condition following the removal or the atrophy of the thyroid, the sugar tolerance is increased. Here we find that the feeding of large quantities of sugar does not lead to glycosuria. When the adrenals are diseased (Addison's disease) the blood sugar falls to a low value; on the contrary, as we have already seen, the injection of adrenalin is very potent in causing the break-down of glycogen. It is evident that in the normal animal the endocrine glands produce a state of perfect balance, those concerned with the breaking down of glycogen being opposed by those concerned with its formation.

Treatment of Diabetes

Up to the time of the discovery of insulin the treatment of diabetes was almost entirely dietetic and consisted in restricting the intake of carbohydrate, protein and fat. The main object of this restriction of the diet was to give an opportunity to those cells of the sugar factory that were out of order to recover some of their lost power. Allen has demonstrated that the high blood sugar damages the pancreas in slightly diabetic dogs. If the diet of the diabetic is not restricted the condition of the patient becomes progressively worse, until finally the power to oxidize glucose is lost entirely. In the Allen treatment the patient is starved until the urine is found to be sugar-free, the necessary time for this being usually three or four days. Then small and gradually increasing amounts of carbohydrates are given, proteins are next added to the dietary in increasing amounts, and finally fats are added. Considerable use is made of green vegetables, such as lettuce, spinach, turnips, etc., which contain but little nutriment.

Whenever sugar reappears in the urine the days of fasting are repeated. Alcohol can be oxidized by the diabetic, and this is given in severe cases in the form of whiskey.

In older methods of treatment considerable quantities of fat and protein were fed, carbohydrates alone being omitted entirely. But it can be seen that feeding protein means that carbohydrate will be produced from this protein, and that feeding fat will increase the amount of acetone bodies formed.

Chapter III

ACIDOSIS

The most serious complication of diabetes mellitus is the formation of acetone bodies. As we have already seen, acetone bodies are produced in normal persons when carbohydrate food is omitted from the diet. In starvation, acetone bodies are formed in considerable amounts, during the entire period. In severe diabetes large quantities of acetone bodies are formed. This ketosis is diminished by the feeding of carbohydrates if some sugar can be oxidized, but not all in the last stages of the disease. Autopsies performed after death from diabetes show the presence of acetone bodies in all tissues; but in the largest amounts in the liver. Acetone bodies fed to normal individuals in moderate amounts are oxidized to carbon dioxide and water; but in the diabetic, acetone bodies cannot be oxidized, and they unite with the alkali of the body causing the condition known as acidosis. Acidosis may be defined as the condition where there is a diminution of the alkaline reserve.

Experimental acidosis can be caused by feeding animals with mineral acids (HCl, HNO_3, H_2SO_4). The normal animal is no more able to oxidize these inorganic acids than the diabetic animal is able to oxidize acetone bodies.

If we feed a normal animal such organic acids as tartaric, citric, or malic, no acidosis will be caused, because unless excessive quantities are fed these organic acids will be completely oxidized to carbon dioxide and water. We find tartaric, citric, and malic acids, and their acid potassium salts in grapes, citrus fruits, apples, and many other fruits. The acid potassium salts, after oxidation by the animal, yield an alkaline ash consisting largely of potassium bicarbonate, and they consequently make the urine more alkaline.

Effects of Acidosis

The acetone bodies, with the exception of acetone itself, which is not an acid, are poisonous because they unite with the bases in the body, forming salts. The presence of sodium bicarbonate in the blood is necessary for respiration, being concerned with the transportation of carbon dioxide from the tissues to the lungs. When a considerable amount of this alkaline reserve ($NaHCO_3$) has been used up for the neutralization of acids, carbon dioxide accumulates in the tissues. The patient passes into a state of coma, dying from a negative asphyxia. The reaction of the blood under normal conditions is very faintly alkaline (pH=7.35–7.45), and we find one part of H_2CO_3 to every 20 parts of $NaHCO_3$. If acids neutralize one-half of this bicarbonate the reaction of the blood will not change toward the acid side because the respiratory center will be stimulated and the more rapid respiration will decrease the amount of carbonic acid in the blood until the ratio of one part of carbonic acid to twenty parts of bicarbonate again obtains. (Haldane believes that a decrease in the pH of the blood of one-tenth of a unit will double the rate of respiration.) But with increasing losses of the alkaline reserve there comes a time when so little bicarbonate is left that no amount of respiration can avail. Here the reaction of the blood may become less alkaline. Just before death the reaction of the blood may become neutral, or even faintly acid.

The animal body combats acidosis in three ways: by oxidizing the acids when possible, by producing a more acid urine, and by forming ammonia to neutralize the acids.

The power of the kidneys to remove weakly acid substance from the blood is considerable. Acid phosphates and weak organic acids are secreted, but more strongly acid substances cannot be eliminated by the kidneys without previous neutralization. The cells produce ammonia to neutralize acids. According to Nash and Benedict, this

formation of ammonia is brought about in the kidney. According to the recent work of Bliss, it occurs in all organs.

In acidosis a considerable amount of acid is neutralized in this manner. Under normal conditions small amounts of phosphoric and sulphuric acids are constantly being formed within the organism. These are formed mainly by the oxidation of the sulphur and the liberation of the phosphoric acid present in proteins and phosphatides. Since the animal produces ammonia to neutralize these two acids, ammonium salts are normally present in the urine. The ammonia nitrogen in urine ordinarily comprises from 3 per cent to 5 per cent of the total nitrogen present. In severe acidosis the ammonia nitrogen in the urine may amount to as much as 40 per cent of the total nitrogen. The estimation of ammonia in urine is of value as a means of detecting a condition of acidosis, provided, however, that the kidneys are functioning properly. In nephritis, acidosis may exist without evidence of this fact being shown by urine analysis. The acidosis of nephritis is possibly due to an inability of the kidneys to secrete acids or salts of these acids, or to produce ammonia. In cases of this sort the urine may be only slightly acid even though the acidosis is severe.

Diagnosis

One of the best means of detecting a state of acidosis is by determining the combining power of blood plasma for carbon dioxide. 100 cc. of normal plasma will combine with about 65 volumes of carbon dioxide. In acidosis this value is often found to have decreased to one-third of the normal value; in fatal cases of acidosis the carbon dioxide combining power decreases practically to nothing.

Van Slyke Method.—The estimation of the combining power of blood plasma for carbon dioxide is best carried out by the Van Slyke method, using the Van Slyke appara-

tus. The method consists, in brief, in collecting blood in a tube containing oxalate and paraffin oil. The oxalate prevents clotting and the oil prevents the escape of carbon dioxide. The corpuscles are now removed by centrifuging and the clear plasma is pipetted into a separatory funnel. The operator now breathes once through a bottle of glass beads and through the funnel. This is to fill the funnel with an atmosphere containing carbon dioxide at approximately normal venous tension. The funnel is now turned end-over-end to saturate the plasma with carbon dioxide at a normal tension. One cubic centimeter of plasma is now placed in the Van Slyke apparatus and subjected to a Torricellian vacuum, acid having been added to release the carbon dioxide from its combination. Upon raising the mercury column the volume of carbon dioxide is read at atmospheric pressure and by reference to tables by Van Slyke the cubic centimeters, reduced to O° C. and 760 mm. pressure bound by 100 cc. of plasma are obtained. It is necessary in the determination to know the temperature of the room and the barometric pressure.

Other Methods.—Other methods for the diagnosis of acidosis are: I. Determination of the amount of carbon dioxide in alveolar air (Fridericia), II. Determination of the titratable alkalinity of the blood, or of plasma. The method of Greenwald and Lewman for the titration of the alkalinity of the blood consists in precipitating the proteins by adding a known volume of a picric acid solution to a known volume of laked blood. The filtrate contains an excess of picric acid, together with the bases of the blood, which are present as picrates. The free picric acid is determined by titration; the total of free and combined picric acid is determined by precipitation with nitron, washing, drying, and weighing. The difference between the total and the free picric acid represents the combined picric acid. Knowing the titration value of the picric acid solution, one

can calculate the amount that has reacted with the bases of the blood and can obtain the value for the alkalinity of the blood in cubic centimeters of tenth normal alkali per 100 cc. of blood.

It might be thought that the animal body would be capable of producing enough ammonia to neutralize all of the acid formed during acidosis, but this is not the case. As a therapeutic measure sodium bicarbonate may be given, usually per os, and in extreme cases intravenously. Besides neutralizing acids the bicarbonate has the effect of preventing the breaking down of glycogen in the liver. While bicarbonate therapy is of some use in the treatment of the acidosis of nephritis, it is not nearly as effective as insulin for diabetic acidosis. When approximately one teaspoonful of sodium bicarbonate is ingested by a normal person, the urine becomes alkaline in reaction within two or three hours, and the neutralization ammonia of the urine practically disappears. Where acidosis is present much larger quantities of bicarbonate must be given to render the urine alkaline, in some cases as much as two hundred grams.

Alkalosis

It is important to make certain by estimating the carbon dioxide combining power of the blood that acidosis really exists before giving bicarbonate. Myers and Booher have pointed out that the condition of alkalosis, or an increased alkalinity of the blood, is much more common than was formerly thought. When the rate of respiration is increased, as in fever, during a very hot bath, or because of residence at great altitudes, so much carbon dioxide is lost by over-ventilation of the lungs that the blood becomes more alkaline. The kidneys endeavor to compensate for this increased alkalinity by secreting an alkaline urine and eventually the alkali reserve will fall below normal and the reaction of the blood will decrease to the normal value.

Van Slyke has described nine different conditions of the blood with respect to its reaction and its carbon dioxide combining power where the pH may be high, low, or normal and the carbon dioxide combining power may be high, low, or normal.

Immediately after the ingestion of sodium bicarbonate the pH of the blood is high (increased alkalinity) and the carbon dioxide combining power is also high. In fever, where there is an undue ventilation of the lungs, the pH is high and the carbon dioxide combining power is low. When a small amount of bicarbonate has been fed over a long period of time the pH may be normal and the carbon dioxide combining power may be high. In incipient acidosis, where there is compensation by more rapid respiration, the pH may be normal even though the carbon dioxide combining power of the blood is low. When the respiration is interfered with and carbon dioxide accumulates in the blood and tissues the pH may be low while the carbon dioxide combining power is either high, or normal. In the terminal stages of acidosis both the pH and the carbon dioxide combining power are low.

Chapter IV

PROTEINS

Proteins comprise a third great class of organic compounds present in animals and plants, and occurring as constituents of foodstuffs. Proteins are found in the branches, roots and leaves of plants in small amounts; but in seeds, large quantities of protein are found. In animals a large part of the body is composed of protein. Protein has always been considered to be the most important constituent of living protoplasm. Proteins occur in the "dissolved" state in plant sap, in blood, lymph, milk and pervading the tissues; as jellies, in a considerable portion of animal tissue; and as solids, in buds and seeds.

Elementary Composition

Proteins are found to contain carbon, hydrogen, oxygen and nitrogen. Sulphur is almost always present. Phosphorus is rather frequently found to be a constituent of the protein molecule. Rarely we find iron (in hemoglobin), copper (in hemocyanin), iodine (in thyreoglobulin), silicon (in the keratin of feathers), and possibly still other elements. The percentages of the more important elements are given below:

carbon	50-55 per cent
oxygen	21-24 per cent
nitrogen	15-18 per cent
hydrogen	6.5-7.3 per cent
sulphur	< 2.2 per cent
phosphorus	< 1.7 per cent

Proteins can be detected by burning and observing the odor similar to burnt hair. Carbonization shows the presence of carbon. Heating with lime, or alkali, develops

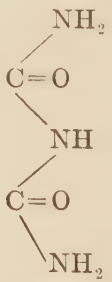
ammonia (from amide groups), showing the presence of nitrogen.

If we heat dry protein with metallic sodium, sodium cyanide, sodium phosphate and sodium sulphide will be formed. Upon dissolving the fused material in water we can detect the nitrogen, phosphorus and sulphur by simple qualitative tests.

Color Tests for Proteins

There are four important color tests more or less characteristic of proteins.

The biuret test is performed by adding sodium hydroxide and a few drops of a very dilute copper sulphate solution to the protein in substance, or in solution. If protein is present, a lavender color develops. All proteins give this test. Peptones give a reddish rather than a violet color. The insoluble keratins should be boiled with hydrochloric acid before applying the test. The biuret test is due to the presence in the molecule of imide groups, $-C=ONH-$, and also amide groups, $-C-ONH_2$. Usually a compound must contain three or more of these groups to give the biuret test. The test is given by the substance biuret and by similar substances.



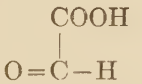
biuret

Millon's Test.—When protein solutions are heated with *Millon's reagent* (a solution of mercuric nitrate and nitrite),

a white precipitate is formed which quickly turns brick red. This test is given only by those proteins which contain a hydroxylated benzol ring. Phenol, as would be expected, gives an intense red coloration with Millon's reagent upon heating.

Xanthoproteic Test.—When proteins are heated with strong nitric acid, the solution is colored yellow. Upon neutralizing with ammonia, an orange color develops. This is the xanthoproteic test, and depends upon the presence of the benzol ring. The colored substance formed is said to be xanthoproteic acid.

Hopkins and Cole Test.—If one adds to a protein solution some glyoxylic acid and subsequently allows concentrated sulphuric acid to form a layer below, a purple ring is formed if the protein in question contains the indol group:



glyoxylic acid

indol

This is known as the Hopkins and Cole test.

Precipitation Tests

There are a great many reagents which precipitate proteins from solution. Some proteins can be precipitated by shaking, or precipitate spontaneously when allowed to stand for some time. The alkaloidal reagents, so called because they form precipitates with the alkaloids, have been extensively used as protein precipitants. Some of these are: picric acid, tannic acid, phosphotungstic acid, phosphomolybdic acid, hydroferrocyanic acid, metaphosphoric acid, sulphosalicylic acid, dinitrosalicylic acid and potassiomercuric iodide. It is necessary in using some of these reagents

to add also acetic acid and sometimes hydrochloric acid for complete precipitation to occur. We can say that when the reagent is added to the protein solution, an insoluble protein salt (e. g., protein picrate or tannate) is formed. From the standpoint of physical chemistry, we can consider that in an acidified solution the colloidal protein particles adsorb hydrogen ions, becoming positively charged. These positively charged particles then unite with negatively charged picrate or tannate ions, and precipitate out. Both of these processes (chemical and physical) probably occur.

Nearly all of the heavy metals, when added in the form of their salts to protein solutions, are capable of precipitating the protein. An excess of the salt may cause this precipitate to redissolve, however. The precipitation of proteins by the heavy metals is usually favored by a neutral, or slightly alkaline reaction. When a copper salt or a lead salt is added to a protein solution, we can say that insoluble copper proteinate or lead proteinate is formed. From the standpoint of colloid chemistry, we can say that negatively charged colloidal protein particles form adsorption complexes with the positively charged metallic ions. It is easy to understand why salts of the heavy metals have a harmful action when taken into the body, and why egg-white is used as an antidote. When proteins are treated with the alkaloid reagents, or with the salts of the heavy metals, the precipitation is often irreversible. The protein has become chemically changed in the process.

Upon the addition of alcohol, proteins are precipitated. This precipitation is reversible if the protein is not allowed to stand in contact with the alcohol for too long a time. Upon long standing alcohol converts protein into a modification which cannot be dissolved in water.

Proteins can be reversibly precipitated by adding to their solutions large quantities of certain soluble salts such as ammonium sulphate, magnesium sulphate, or sodium sul-

phate at 30° C., and in some cases sodium chloride. Of these salts, ammonium sulphate is the one most often used. All protein is precipitated when its solution is saturated with ammonium sulphate. This method of precipitation is known as "salting out" and is of value when it is desirable to concentrate protein from a dilute solution. "Salting out" is often used as a means of separating one protein from another, inasmuch as some proteins are more readily precipitated than others.

In some instances proteins are precipitated by the action of enzymes. Thus, rennin converts casein of milk into the insoluble paracasein. The insoluble product is a calcium salt.

Proteins are readily precipitated by the addition of suspension colloids, provided the charge on the colloidal particles is opposite in sign to that on the protein particles. Colloidal ferric hydroxide and aluminum hydroxide are frequently used to remove protein from solution, especially in cases where the protein has an interfering action in the analysis of some substance present.

Most proteins are precipitated upon heating their solutions to 60–80° C. This process is irreversible, and is called heat coagulation. In alkaline or neutral solutions the coagulation either does not take place or else is incomplete. For complete coagulation and precipitation of protein, the presence of salt and a small quantity of acid is necessary. The acid, which must not be present in too great amount, assists in the conversion of the protein from an *emulsion colloid* to a *suspension colloid.* The salt causes a flocking out of the particles of the suspension colloid.

In all cases where proteins are precipitated we have a transformation from the hydrosol to the hydrogel state. Certain proteins are not coagulated by heat. Gelatin is an example. Upon heating with water, gelatin passes to the

hydrosol state. Upon cooling, gelatin forms a hydrogel if the solution is not too dilute.

Proteins are precipitated by strong acids at certain concentrations. Upon this fact depends Heller's test for protein. The solution to be tested is underlaid with strong nitric acid, care being taken not to mix the two liquids. At a certain zone of acid concentration a white ring of precipitated protein will be seen if protein is present. One part of protein in 60,000 parts of water can be detected by this test.

It is often important to test urine for protein. The best tests for the physician are Heller's, the heat coagulation, the sulphosalicylic and the dinitrosalicylic tests. The latter two tests are to be preferred since they are more trustworthy in unskilled hands. With dinitrosalicylic acid it is possible to detect one part of protein in 1,000,000 parts of water, provided the test is observed against a bright beam of light in a dark room. Urine that is turbid must be clarified by filtration before testing.

Classification of Proteins

Below is given the classification of the proteins as adopted by the American Society of Biological Chemists. The native (unaltered) proteins, which are classified as simple proteins, are those that are not united with other groups (prosthetic groups) such as hematin, sugars, nucleic acids, and the like. The derived proteins, which are discussed on page 103, are proteins that have been altered by the action of heat, enzymes, or chemical reagents. The classification is not satisfactory in every respect. Egg albumin, for example, contains phosphorus and might well be placed in the group of phosphoproteins. Again, under the heading "albuminoids" one finds a most diverse assemblage of proteins with widely different properties.

Classification of Proteins

Simple Proteins	*Conjugated Proteins*	*Derived Proteins*
albumins	nucleoproteins	proteans
globulins	glycoproteins	coagulated proteins
glutelins	phosphoproteins	metaproteins
prolamines	hemoglobins (or chromoproteins)	proteoses
albuminoids (or scleroproteins)	lecithoproteins	peptones
histones		
protamines		

General Properties of Proteins

When in the solid state, the proteins are usually faintly yellow, amorphous substances (some plant globulins, some animal albumins, and the hemoglobins are crystallizable). With small amounts of water the proteins form gels and with larger amounts of water they form sols. The proteins are odorless, tasteless and, with the exception of the hemoglobins, nearly colorless. Protein solutions are colloidal and are levorotatory, with the exception of some of the conjugated proteins. The protamines and histones are basic in reaction, while the other proteins are slightly acid.

Of the simple proteins, the albumins, histones, and protamines are soluble in pure water. The globulins are soluble in dilute neutral salt solutions, but are insoluble in distilled water. The prolamines can be dissolved in 60–70 per cent alcohol. The glutelins and phosphoproteins dissolve in dilute alkali and acid, and are reprecipitated after neutralizing. The albuminoids are, for the most part, only slowly soluble in strong acids, or alkalis. Some albuminoids (sericin) and albuminoid derivatives (gelatin) dissolve in hot water but merely swell up in cold water.

Albumins.—Albumins are soluble in pure water, coagulate on heating, and are (animal albumins) salted out only by saturating with ammonium sulphate. They contain considerable amounts of sulphur, but no glycocoll. Examples are: two serum albumins in blood, one of which can

be crystallized; myogen, in muscle, lactalbumin in milk, and ovalbumin and conalbumin in hen's eggs. Ovalbumin is said to contain phosphorus.

Vegetable albumins are found in plants only in small amounts. Some are: leucosin in wheat, rye and barley; legumelin in peas, soy beans and horse beans; phaselin in kidney beans, and ricin in castor beans. This last is a toxalbumin—one part intravenously injected is capable of killing two million parts of rabbit. Some of the vegetable albumins are precipitated by half saturation with ammonium sulphate.

Globulins.—Globulins are characterized by their insolubility in pure water, and their solubility in the presence of neutral salts. The animal globulins are precipitated by half-saturation with ammonium sulphate and by complete saturation with magnesium sulphate. The globulins are coagulated by heat. The plant globulins are not always precipitated by half saturation with ammonium sulphate, and are often difficult to coagulate by heat. Some of the globulins in legumes act like globulins with respect to solubility only after the addition of a trace of acid. Examples of animal globulins are eu-globulin, pseudo-globulin and fibrinogen of blood plasma. In muscle we find myosin, which coagulates like fibrinogen on standing. Globulins are present in nervous tissue and a globulin occurs in milk. There is much doubt as to just how many different globulins may be present in animal tissues.

The vegetable globulins are very numerous and comprise the greater part of the protein content of most seeds. A number of these globulins are capable of crystallizing. Examples of crystallizable plant globulins are: edestin in hemp seed, excelsin in Brazil nuts and concanavalin A and concanavalin B in the jack bean. Figure 2 shows bisphenoidal crystals of concanavalin A and figure 3 shows hexagonal needles of concanavalin B.

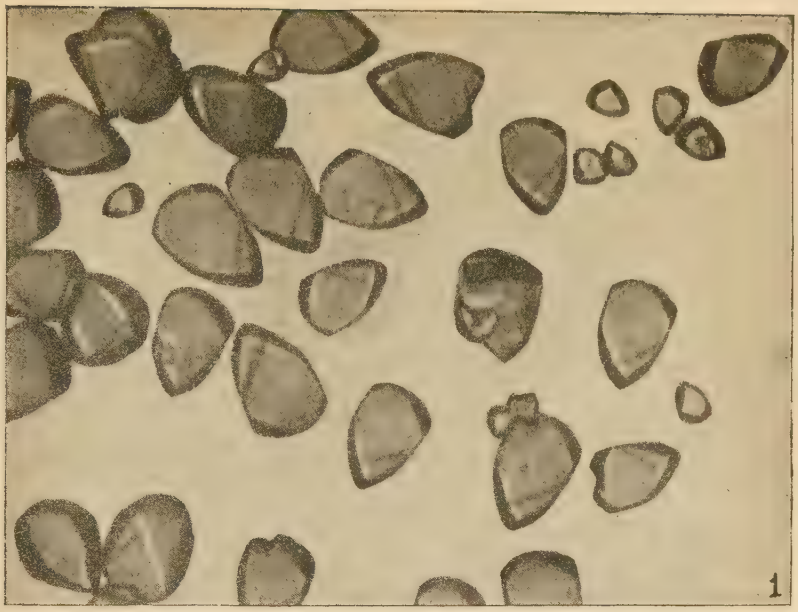


FIG. 2. Globulin crystals: Concanavalin A (magnified 150 diameters).
[Reproduced from *Jour. Biol. Chem.*]

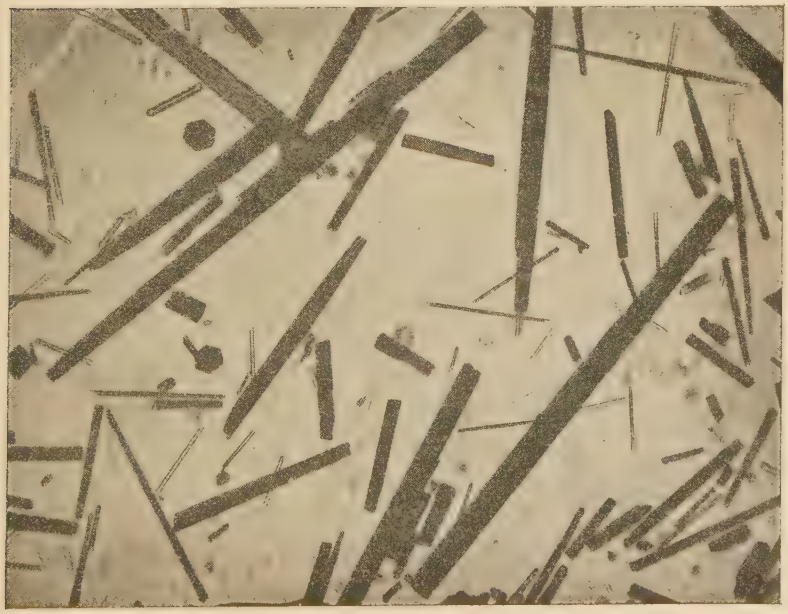


FIG. 3. Globulin crystals: Concanavalin B (magnified 135 diameters).
[Reproduced from *Jour. Biol. Chem.*]

Other plant globulins are: tuberin in potatoes, legumin in peas, lentils and vetches; amandin in peach and almond seeds and canavalin in the jack bean.

Glutelins.—Glutelins are not found in animals and seldom occur in plants. Glutelins are characterized by their insolubility in pure water and salt solutions and by their solubility in dilute acids and alkalis. They have been called vegetable caseins on this account. The most important of the glutelins is glutenin of wheat. This is probably also present in barley and rye. The glutelin of rice is called oryzenin. These glutelins are very important food proteins.

Prolamines.—Prolamines, or gliadins, are found only in plants. They are insoluble in distilled water and in salt solutions but dissolve in 60–70 per cent alcohol. Dilute acids and alkalis also dissolve them. It should be mentioned that while insoluble in water, both the glutelins and the prolamines swell upon the addition of water, forming gels. This makes it possible to form a dough with wheat flour. Upon heating these gels (as in baking bread), the proteins coagulate. Examples of the prolamines are: gliadin in wheat and rye, zein in corn and hordein in barley.

Albuminoids.—Albuminoids, as the name indicates, have been looked upon as substances similar to the true proteins, but not identical with them. Instead of the term albuminoids, these proteins are sometimes called sclero-proteins, signifying their prominence in skeletal tissues. The albuminoids are usually insoluble in all solvents save strong alkalis and acids. They are very resistent to micro-organisms, and most of them are indigestible. Classified as albuminoids, we find proteins very dissimilar in properties and in amino acid make-up. The albuminoids are not found in plants, their place being occupied by cellulose. Examples of albuminoids are: keratins, elastin, collagen, reticulin, spongin, fibroin and sericin.

Hair, wool, skin (epidermis), nails, hoofs, horns and feathers are impure *keratins.* Neurokeratin is present in brain and nerves. The membrane within the egg shell is keratin. Keratins are indigestible; they contain much sulphur (cystin).

Elastin is an albuminoid found in elastic tissue, in ligaments and in the walls of arteries. Elastin is digested by enzymes.

Collagen is an albuminoid present in skin (corium), bone and cartilage. The jelly-like structure which remains after the mineral matter of bone has been dissolved in hydrochloric acid, is mostly collagen. Collagen is digested by the gastric juice, but is not digested by trypsin in its native state. When collagen is boiled for some time in water, it is hydrolysed to form gelatin, which goes into solution. This change is said to be reversible. The characteristic properties of gelatin are its ability to imbibe cold water, whereby it forms a gel, but does not dissolve, and its ability to dissolve in warm water (30° C.) and to form a gel again upon cooling. Upon long boiling gelatin is hydrolysed to a substance which will no longer gel. Gelatin (and consequently collagen) contains no tryptophan and only traces of cystin and tyrosin. It should be classified as a derived protein since it is not found in nature.

Reticulin is an albuminoid present in lymphatic glands, in the spleen, liver, kidneys, lungs and other organs. It is indigestible and is insoluble in dilute acids and alkalis. Reticulin contains phosphorus.

Spongin, the skeleton of the sponge, is soluble in strong acids and alkalis. It contains iodo-tyrosin.

Fibroin and *sericin* are the albuminoids of silk. The sericin, or silk gelatin, is dissolved by treating the raw silk with boiling water, leaving behind the fibroin, which is the part used. Spider silk contains no sericin.

Histones.—Histones are basic proteins of relatively simple structure. They contain little or no sulphur and only small amounts of tyrosin. The histones are found in spermatozoa in combination with nucleic acids as nucleoproteins. Histones are not found in plants. Examples are: scombron in mackerel spermatozoa, gadus histone from the codfish and lota histone from the burbot. The conjugated protein hemoglobin contains the histone globin in combination with hematin. The histones are soluble in water and are not coagulated by heat.

Protamines.—The simplest proteins are the protamines. They contain few varieties of amino acids and are composed largely of the hexone bases. On this account they are more basic than the histones. Like the histones they occur in combination with nucleoproteins in the heads of spermatozoa, from which they can be prepared by extracting with 2 per cent sulphuric acid. There are no vegetable protamines. Examples are: salmine from salmon, sturine from the sturgeon, scombrine from mackerel, cyprinine from carp and clupeine from herring.

Amino Acids

When proteins are boiled for fifteen or twenty hours with 35 per cent hydrochloric acid, or 20 per cent sulphuric acid, they are converted into their "building stones" the amino acids, which correspond to the monosaccharides that are formed from polysaccharides. The usual test showing that the hydrolysis is complete is the biuret test. When the biuret test is no longer given, the product is said to be abiuret. The Van Slyke method for the determination of amino nitrogen, which is described on page 101, is more reliable than the biuret test. If further boiling with acid causes no increase in the amino nitrogen, as determined by the Van Slyke method, the hydrolysis can be said to be

complete. Acid hydrolysis of proteins produces mainly amino acids, but other substances, such as amino acid anhydrides, ammonia and humin are formed. The humin is a complex pigment that is black in color. It appears to be formed largely by the action of the hot acid upon one of the amino acids, tryptophane.

Instead of using acids one can use an enzyme, such as trypsin, to hydrolyse proteins, but when trypsin is used the process takes a longer time and does not go to completion. Alkali is not usually used for the hydrolysis of protein, for alkali destroys many of the amino acids, forming fatty acids, sodium sulphide and ammonia. Barium hydroxide is used as a hydrolytic agent in certain cases.

Besides occurring in proteins in the combined state, amino acids are found in the free state in very small amounts in the intestinal contents during protein digestion, in blood, urine, in germinating seeds and in the soil. The amino acids are colorless substances, crystalline (with one exception) and readily soluble in water (with two exceptions). Some of the amino acids are bitter in taste, others are sweet.

At least twenty amino acids are known to occur in nature. One of these is optically inactive, the others are optically active. The amino acids, with two exceptions, are characterized by the presence of a carboxyl group and an alpha amino group; in other words, they are alpha amino acids. Some of the amino acids have two amino groups, and but one carboxyl and consequently possess a predominantly basic character; other amino acids possess two carboxyl groups are only one amino group and are more acid in nature. The amphoteric nature of the amino acids is probably what makes them of such great value to the organism, they are both acid and basic, but only feebly so.

Glycocoll, or glycine (amino acetic acid) is the simplest amino acid. The name glycine means sweet glue, glycine

being a sweet substance first prepared from glue, or gelatin, in which it occurs to the extent of 20 per cent. Glycocoll is not found in casein, hemoglobin, or in the albumins. Glycocoll can be prepared as follows by hydrolysing hippuric acid:

CH_2NH_2
|
COOH
glycocoll

$$\underset{\text{hippuric acid}}{C_6H_5CONHCH_2COOH} + H_2O = \underset{\text{benzoic acid}}{C_6H_5COOH} + \underset{\text{glycocoll}}{CH_2NH_2COOH}$$

Glycocoll can be synthesized in two ways, which are applicable to the synthesis of many other amino acids. The first of these methods consists in treating a halogen derivative of a fatty acid with ammonia:

$$\underset{\text{monochlor acetic acid}}{CH_2ClCOOH} + HNH_2 = \underset{\text{glycocoll}}{CH_2NH_2COOH} + HCl$$

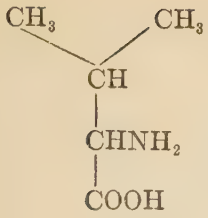
The second method, which is known as the Strecker synthesis, is as follows:

$$\underset{\text{formaldehyde}}{HC(=O)H} + \underset{\text{ammonia}}{HNH_2} + \underset{\text{hydrocyanic acid}}{HCN} = \underset{\text{methylene cyanamine}}{CH_2NH_2CN} \xrightarrow{\text{hydrolysis}} \underset{\text{glycocoll}}{CH_2NH_2COOH} + NH_3$$

It will be seen that glycocoll contains no asymmetric group and is therefore optically inactive.

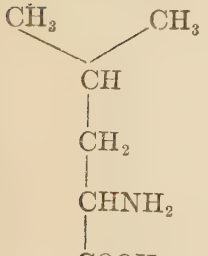
CH_3
|
$CHNH_2$
|
COOH
d. alanin

d. Alanin (alpha amino propionic acid) is found in proteins in small amounts. It is best prepared from silk (fibroin). It is dextro-rotatory.



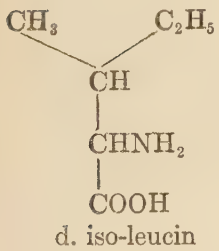
d. valin

d. Valin (iso-propyl glycocoll) is present in casein, edestin and keratin.



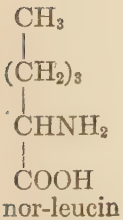
d. leucin

l. Leucin (iso-propyl alanin) is present in casein and hemoglobin. In the free state it is found in the perspiration of the feet and in small amounts in animal tissues. It decomposes readily, forming iso-amyl alcohol. When pure, leucin is rather insoluble in water. Leucin is the second most common amino acid.



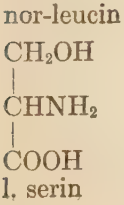
d. iso-leucin

d. Iso-leucin (ethyl-methyl-alanin) is best prepared from beet-sugar molasses.



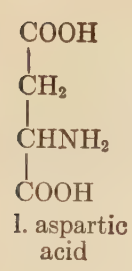
nor-leucin

Nor-leucin (alpha amino-caproic acid) has been obtained from the brain by Abderhalden, but has not been since reported as a constituent of the proteins.

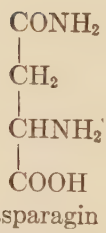


l. serin

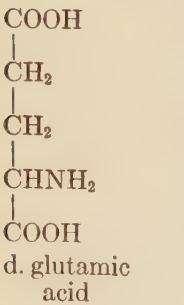
l. Serin (beta hydroxy alanin) is present in sericin (silk gelatin) and in salmine.


l. aspartic acid

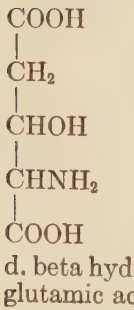
l. Aspartic acid (amino-succinic acid) has been found in the free state in the slime of sea snails. It occurs in asparagus and many other plants in the free state as the amide, asparagin:


asparagin

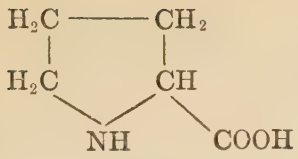
The aspartic acid obtained by hydrolysing proteins is probably produced, together with ammonia, by the action of the acid upon the asparagin originally present.


d. glutamic acid glutamine

d. Glutamic acid (glutaminic acid, or alpha amino-glutaric acid) comprises nearly 40 per cent of the gliadin of wheat. It probably exists in proteins as the amide, glutamine.

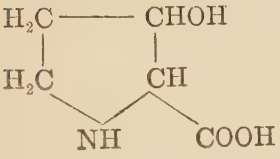

d. beta hydroxy glutamic acid

d. Beta hydroxy glutamic acid has recently been isolated from casein, gliadin and glutenin.



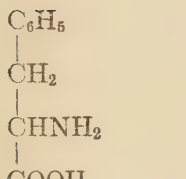
l. prolin

l. Prolin (alpha pyrollidine carboxylic acid) is present in salmine and in other protamines. It has a sweet taste.



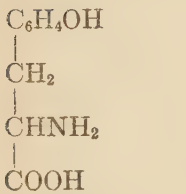
oxyprolin

Oxyprolin possibly has this structural formula. It is present in gelatin and casein.



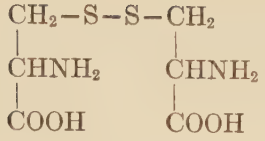
l. phenylalanin

l. Phenylalanin was first discovered in lupin seedlings in the free state. It has a bitter taste. Phenylalanin is one of the amino acids responsible for the xanthoproteic test.



l. tyrosin

l. Tyrosin (para hydroxy phenylalanin) is the amino acid responsible for Millon's test. It was discovered many years ago by Liebig, who prepared it by fusing cheese with potassium hydroxide. Tyrosin forms pigments of reddish and black color upon oxidation by the enzyme tyrosinase, and is thought to be one of the amino acids responsible for animal pigments. Tyrosin may possibly be the mother substance of the hormone adrenalin.

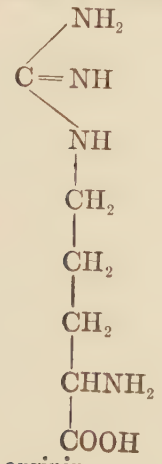


l. cystin

l. Cystin (di beta thioalanin) occurs in large amounts in hair and wool (keratin). It is destroyed by heating with alkali, which splits off the sulphur. Cystin is the amino acid which is responsible for the sulphur test given by proteins. Not all of the sulphur present in proteins is cystin sulphur. Upon reduction, cystin is converted into cystein (beta thioalanin). In the metabolic derangement known as cystinuria cystin from the endogenous metabolism passes into the urine, although cystin in the diet is readily oxidized. Cystin is one of the insoluble amino acids. Urinary calculi are sometimes composed of cystin. Cystin crystallizes readily in six-sided plates.

cystein

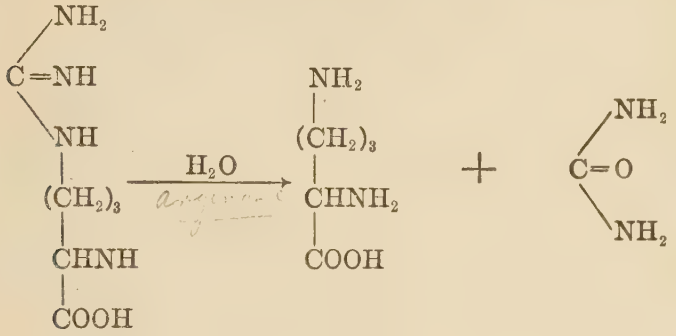
A new amino acid containing sulphur has recently been discovered in casein by Mueller. This amino acid contains sulphur bound in such a manner that it is not split off by heating with sodium hydroxide. Its empirical formula is $C_5H_{11}SNO_2$.



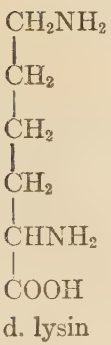
d. arginin

d. Arginin (alpha amino-delta guanidin-valerianic acid), together with lysin and histidin (q. v.), has been called a hexone base because of its basic nature and because it contains six carbon atoms. It is the amino acid most widely distributed in proteins. Arginin is a diamino acid, as it contains two amino groups. By the action of

the enzyme arginase, or by heating with strong alkali, the guanidin group in arginin is split off, yielding urea and ornithin, the reaction being as follows:

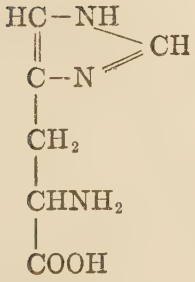


d. arginin ornithin urea



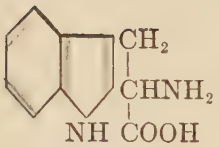
d. lysin

d. Lysin (alpha epsilon-diamino-caproic acid) is another of the hexone bases. Lysin is non-crystalline, having been obtained as a liquid. It can be prepared from casein, or gelatin.



l. histidin

l. Histidin (beta imidazole-alanin), the third of the hexone bases, contains the imidazole ring. It was first discovered in sturine.



l. tryptophan

l. Tryptophan (beta indol-alanin) is destroyed by the acid hydrolysis of proteins, but not by hydrolysing with barium hydroxide. Tryptophan is the amino acid in proteins responsible for the Hopkins and Cole test. Tryptophan contains the indol ring. The indol ring is present in indigo and other important dyes. The decomposition products of tryptophan, indol and skatol are responsible for the bad odors of putrefying organic matter.

The accompanying table (page 99) shows the percentages of the various amino acids that have been isolated from some representative proteins. With the exception of the figures for salmin, the percentages in this table are all too low because at present we lack adequate means of separating quantitatively all of the amino acids formed by the hydrolysis of proteins. If the percentages in the table were correct, the totals would amount in each case to over 110 per cent, inasmuch as from 10 to 20 per cent of water is added during hydrolysis, depending upon the number and the molecular weight of the amino acids present.

It should be understood that the protein molecule, in the case of the simple proteins, is entirely composed of these amino acids. Some proteins contain no more than four varieties, other proteins contain as many as fifteen varieties. The number of molecules of any one amino acid present in a protein might vary from one to twenty, or thirty. The total number of amino acid molecules in a protein might be anywhere from 50 to 500, depending upon the complexity of the protein. The molecular weights of the proteins are not known. Estimates based on various physical and chemical observations have ranged from

14,000 to as high as 100,000. These figures show how uncertain this matter is at present. Abderhalden has calculated that with only one molecule each of twenty different amino acids nearly 2.5 quintillion different proteins could be theoretically constructed.

PER CENT OF AMINO ACIDS ISOLATED FROM VARIOUS PROTEINS

amino acid	salmin (salmon)(1)	gelatin (2)	ovalbumin (hen's egg) (3)	gliadin (wheat) (4)	zein (maize) (5)
glycocoll	0	25.5	0	0.02	0
alanin	0	8.7	2.22	2.00	9.79
valin	4.3	0	2.50	0.21	1.88
leucin	0	7.1	10.71	5.61	19.55
isoleucin	0	0	.....	.....	.....
serin	7.8	0.4	?	0.13	1.02
phenyl-alanin	0	1.4	5.07	2.35	6.55
tyrosin	.....	0.01	1.77	1.20	3.55
prolin	11.0	9.5	3.56	7.06	9.04
oxyprolin	0	14.1	.....	.....	.....
aspartic acid	0	3.4	2.20	0.58	1.71
glutamic acid	0	5.8	9.10	37.33	26.17
hydroxy glutamic acid	.....	0	.....	.....	.....
histidin	0	0.9	1.71	0.61	0.82
arginin	87.4	8.2	4.91	3.16	1.55
lysin	0	5.9	3.76	0	0
cystin	0	.....	?	.....	.....
tryptophan	.....	.....	+	+	0
ammonia	0	0.4	1.34	5.11	3.64
totals	110.5	91.3	50.08	65.37	84.27

(1) Kossel and Dakin. (2) Dakin. (3) Osborne, Jones and Leavenworth. (4) Osborne and Clapp. (5) Osborne and Liddle.

The Estimation of Amino Acids

The monamino acids resulting from the hydrolysis of proteins can for the most part be estimated by Emil Fischer's distillation method. In this method the hydrolytic

products are converted into ethyl esters and these esters are fractionally distilled in vacuo. The various fractions are then saponified and the resultant amino acids are separated by fractional crystallization. The diamino acids, including also histidine and cystin, can be separated quantitatively from the original hydrolysis mixture by the method of Kossel. This method involves precipitation of the amino acids mentioned above with phosphotungstic acid and precipitation of histidin and arginin with silver sulphate in alkaline solution.

Dakin's Butyl Alcohol Extraction.—Dakin has devised a method for the separation of monamino acids by extraction with butyl alcohol. In this method the protein is hydrolysed with sulphuric acid. The sulphuric acid is removed as barium sulphate and the solution is concentrated. The concentrated neutral solution is now submitted to a process of continuous extraction with butyl alcohol for a period of 36 hours. The monamino acids are extracted, together with prolin and the peptide anhydrides. The diamino and the dicarboxylic acids are not extracted. The extracted monamino acids precipitate from the butyl alcohol as the process of extraction proceeds. Prolin and the peptide anhydrides remain in solution. The peptide anhydrides can be separated from prolin because of their slight solubility in water.

Van Slyke Identification of Proteins.—Van Slyke has worked out a method for the analysis and identification of proteins by which values for the humin nitrogen, amide nitrogen, diamino nitrogen and monamino nitrogen can be obtained, using only small amounts, 1–3 grams of protein. The values for arginin, histidin, lysin and cystin are also obtained. In this method the protein is hydrolysed by boiling with hydrochloric acid. The hydrochloric acid is then removed by distillation under reduced pressure. The amide nitrogen is removed as ammonia by adding calcium hydroxide and alcohol and distilling into a known excess of

acid. The humin nitrogen is estimated separately by the Kjeldahl method after filtering off and washing the humin precipitate. The basic nitrogen, including histidin and cystin, is precipitated by phosphotungstic acid and is estimated by the Kjeldahl method. An estimation of the sulphur present in the precipitate by phosphotungstic acid enables one to calculate the amount of cystin. An estimation of the ammonia produced by boiling an aliquot of the precipitated bases with concentrated alkali gives figures from which the quantity of arginin can be calculated. The monamino acids are estimated in toto by determining the amount of monamino nitrogen in the filtrate from the diamino acids. Monamino nitrogen is determined by means of an apparatus devised by Van Slyke. The method depends upon the action of nitrous acid on amino groups, whereby nitrogen gas is evolved. This gas is collected and estimated by volume. The reaction is:

$$\begin{array}{lcl} CH_3 & & CH_3 \\ | & & | \\ CHNH_2 & + HONO = & CHOH + N_2 + H_2O \\ | & & | \\ COOH & & COOH \end{array}$$

alanin as one of the amino acids — nitrous acid — lactic acid — nitrogen gas

The Van Slyke method for the determination of amino nitrogen is of value for estimating amino acids en bloc in blood, urine and digests. A determination of amino groups can be used to throw light on the complexity of proteins, or protein decomposition products. In native proteins there are few free amino groups. During hydrolysis more and more of the nitrogen appears as amino nitrogen and when hydrolysis is complete the greater part of the nitrogen exists as amino nitrogen.

Kjeldahl Method.—The total nitrogen in any plant, or animal compound is estimated by the Kjeldahl method.

This important method consists in boiling the material with concentrated sulphuric acid and a catalyst, such as copper sulphate. Potassium sulphate, or phosphoric acid is sometimes added to raise the temperature. The digestion converts all of the nitrogen into ammonium sulphate. When the process of digestion is complete the ammonia is distilled, or aerated off into a known excess of standard acid and is estimated by titrating the excess of acid, or by Nesslerization.

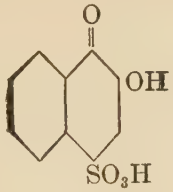
Sörensen Titration of Amino Acids.—Amino acids can be estimated by Sörensen's formol titration. Upon treatment with formaldehyde the hydrogen of amino groups is replaced by the methylene group. This reaction does away with the basic effect of the amino groups and the resulting product becomes acid and can be titrated with sodium hydroxide, or barium hydroxide, using phenolphthalein as indicator. Ammonium salts can be estimated by a similar process. The reaction involved is:

$$\begin{array}{lcl} \mathrm{CH_2NH_2} & & \mathrm{CH_2N{=}CH_2} \\ | & + \quad \mathrm{HC{=}O} \quad = & | \quad + \quad \mathrm{H_2O} \\ \mathrm{COOH} & & \mathrm{COOH} \\ \text{glycocoll} & \text{formaldehyde} & \text{methylene glycocoll} \end{array}$$

Ninhydrin Test.—Amino acids and compounds containing amino groups give a blue color when boiled with triketohydrindene hydrate (ninhydrine). This test is one of the best and easiest to use when it is desired to test qualitatively for the presence of amino groups. Ninhydrine has been used by Abderhalden to demonstrate proteolysis and has been used by Harding and MacLean for the quantitative estimation of amino acids.

Folin Method for Amino Acids.—The method of Folin for the quantitative estimation of amino acids depends upon the colorimetric determination of the red color pro-

duced with the reagent beta naptho-quinone sulphonic acid. This has the following structural formula:



Estimation of Tyrosin, Trytophan and Cystin

Several methods exist for the determination of tyrosin, tryptophan and cystin. The procedure of Folin and Looney will be described as an example. For the estimation of tyrosin and tryptophan the protein is hydrolysed by boiling with a reflux condenser with barium hydroxide. The hydrolysate is then acidified with sulphuric acid and heated to drive off hydrogen sulphide. The tryptophan is now precipitated with mercuric sulphate in 5 per cent sulphuric acid, while the tyrosin stays in solution. The filtrate and the precipitate are treated separately with the Folin and Denis phenol reagent and sodium carbonate solution, which produces an intense blue reduction product. The colored solutions are read in a colorimeter against amino acid standards similarly prepared. For the determination of cystin the protein is hydrolysed with hydrochloric acid, since barium hydroxide destroys the cystin. The hydrolysate is treated with a solution of sodium carbonate and sodium sulphite. The sulphite reduces the cystin to cystein. Upon adding the Folin uric acid reagent (phosphotungstic acid) a blue reduction product is formed by the action of the cystein. The color is read in a colorimeter against a standard similarly prepared from a cystin solution.

Partial Cleavage Products of Proteins

When protein solutions are treated with acid, alkali, heat, or proteolytic enzymes, the protein becomes modified, either

by a process of hydrolysis, or a splitting off from the molecule of certain substances, such as hydrogen sulphide or ammonia. This process is irreversible and the protein is said to have been denatured. If the denaturing has been slight we have formed such products are proteans, meta-proteins and coagulated proteins. If the process of hydrolysis has continued farther, proteoses and peptones are formed.

Proteans are formed from the native proteins by the action of dilute mineral acids, or by standing for a long time in contact with distilled water. *Meta-proteins* result from the action of strong acids, or alkalis. *Coagulated proteins* are formed when a solution of protein is heated. These three classes of derived proteins are not soluble in neutral solution and are known as primary protein derivatives. While the albumins and the globulins are readily convertible into proteans, meta-proteins, or coagulated proteins, certain other kinds of proteins are not. Gelatin, for example, is not affected by dilute mineral acid, or by brief contact with stronger acid and does not coagulate upon boiling.

When the hydrolysis of protein has been carried to some length, the products are readily soluble in distilled water and are known as *proteoses* and *peptones*. The distinguishing characteristic between proteoses and peptones is that the proteoses can be salted out of solution by the addition of ammonium sulphate, while the peptones cannot be salted out. Proteoses and peptones are not coagulated by boiling. They possess a bitter taste and a meat-like odor. They can be compared with the various dextrins that are formed by the hydrolysis of starch. One of the most characteristic tests for proteose is with picric acid, or with other alkaloidal reagents. Picric acid forms a precipitate which dissolves upon heating and reappears upon cooling. The peptones do not give precipitates with the alkaloidal

reagents, but are precipitated with tannic acid and with phosphotungstic acid. While formed by gastric and by tryptic digestion, proteoses and peptones are not present in the blood and if injected into animals have a toxic action and cause what is known as peptone shock. Proteoses are sometimes found in plants.

Much work has been performed in past years in attempts to separate proteoses and to isolate individual compounds by fractional salting out with ammonium sulphate. Proteoses have been separated into fractions, depending on their solubility. Some of the names applied to these fractions are heteroproteose, protoproteose, dysproteose, and deuteroproteose. At the present time it is realized that these terms have little, or no significance and that various fractions of proteoses are complex mixtures that cannot be separated into their components. In a very few instances individual proteoses, or peptones have been isolated after the partial hydrolysis of a protein. Thus, if silk fibroin is allowed to stand in contact with concentrated hydrochloric acid for a number of days an individual peptone can be precipitated from the material with phosphotungstic acid and purified by fractional precipitation with alcohol. This peptone is said to be composed of two molecules of glycocoll, one of alanin and one of tyrosin.

Very little has been learned about the chemical constitution of the proteins by investigation of their partial cleavage products. By using synthetic methods, however, Emil Fischer has accomplished much to make clear the chemical structure of the proteins.

Synthesis of Polypeptides

Starting with amino acids, Fischer and his collaborators have synthesized what they term polypeptides. These are definitely characterized combinations of two, or more, amino acids formed by the elimination of water. The

simpler polypeptides can be compared to the peptones, while the more complex polypeptides exhibit many of the properties of the proteoses.

Fischer synthesized his amino acids by treating various brom-derivatives of fatty acids with ammonia, and also by Strecker's cyanhydrine method (see page 92).

The amino acids produced by these methods were racemic. Fischer could not obtain individual polypeptides starting from racemic amino acids. It can be seen that *d. l.* alanin joined to *d. l.* leucin would give a mixture of the following dipeptides:

d. alanyl d. leucin
d. alanyl l. leucin
l. alanyl d. leucin
l. alanyl l. leucin

Resolution of Racemic Compounds

Pasteur long ago discovered three ways of separating the active components of racemic compounds. These three methods are briefly described below:

Mechanical Method.—In some cases the racemic compound forms both right-handed and left-handed crystals, which are large enough to be picked out by hand and placed in two piles. Pasteur was able to resolve inactive sodium ammonium tartrate in this manner.

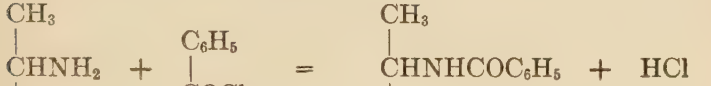
Biological Method.—When a racemic compound is oxidized by an animal, or fermented by a micro-organism, one enantiomorph will be more easily broken down than the other. Common green mould *Penicillium glaucum* attacks dextro-tartaric acid more readily than it attacks levo-tartaric acid.

Fractional Crystallization of Alkaloid Salts.—When d. l. tartaric acid is added to the alkaloid l. chinchonine the following compounds are formed:

l. chinchonine d. tartrate
l. chinchonine l. tartrate

These salts are not related as an object is to its mirror image and accordingly must have different physical properties. The possession of different physical properties will enable one to separate them by fractional crystallization. After resolution the chinchonine can be removed. Other alkaloids that have been used are brucine and strychnine.

Fischer used the method of fractional crystallization of alkaloid salts for resolving his racemic acids, but the amino acids had first to be *benzoylated* so that they would be acid enough to form salts with the alkaloids. The reaction of benzoylation is given below:



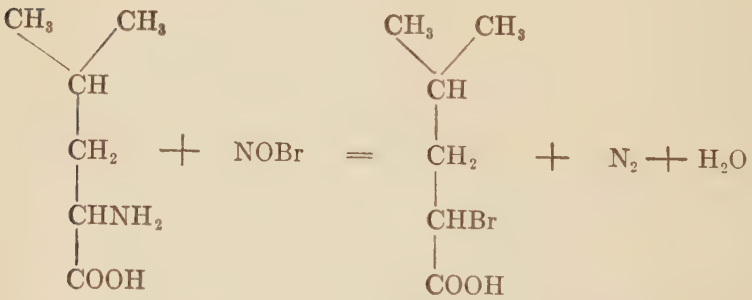
d. l. alanin — benzoyl chloride — d. l. benzoyl alanin

The racemic benzoyl derivative, in this case benzoyl alanin, was next treated with the alkaloid brucine. The brucine salt of l. benzoyl alanin crystallized out, the dextro modification remaining in the mother liquor. The brucine was removed from the mother liquor and by the addition of strychnine the strychnine salt of d. benzoyl alanin was next obtained. By treating these alkaloid salts with alkali, the alkaloids were removed. The benzoyl groups were next split off by means of acid hydrolysis. The reaction is:

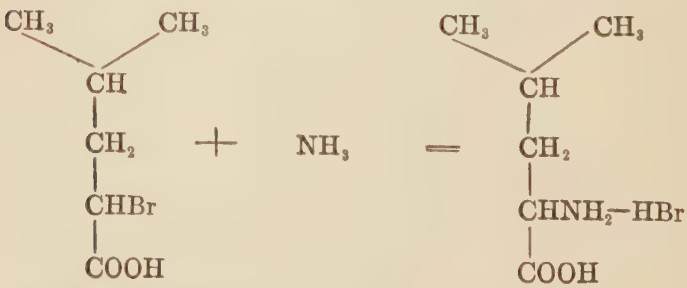
benzoyl alanin — alanin — benzoic acid

Walden Inversion

In order to make as much use as possible of the amino acid optical isomers not found in nature, Fischer converted these into the naturally occurring forms by means of the Walden inversion. By this procedure a levo-rotatory amino acid can be changed into a dextro-rotatory amino acid and a dextro-rotatory amino acid can be changed into a levo-rotatory one. The amino acid is converted into a bromine derivative by treatment with nitrosyl bromide and then, by the action of ammonia, is reconverted into an amino acid. The reason for the change in rotation is not understood. With leucin the reaction is:



d. leucin nitrosyl bromide l. brom-isocaproic acid



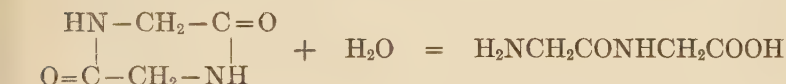
l. brom-isocaproic acid l. leucin hydrobromide

Polypeptides from Amino Acids

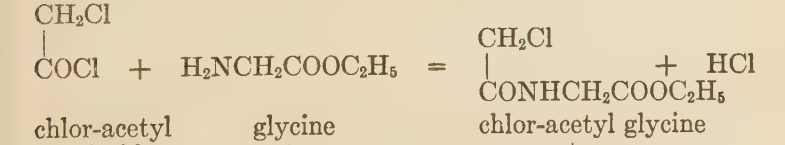
Having at last a stock of amino acids identical with the amino acids occurring in nature, Fischer proceeded to unite these and thus form polypeptides. Of the several methods used only the following two will be considered here:

I. When an ethyl ester of one of the amino acids is heated and in some cases when the ethyl ester is simply allowed to stand in solution, the anhydride, or diketopiperazine is formed:

Upon standing in contact with 11.5 N sodium hydroxide the anhydride ring is opened with the formation of a dipeptide:



II. When the ethyl ester of any amino acid is allowed to react with chlor-acetyl chloride, or with any halogen acyl chloride, the two molecules unite with the formation of an imido group. By gentle hydrolysis the ethyl group is removed and by treatment with ammonia the chlorine atom is replaced by an amino group, giving a dipeptide, in this illustration glycyl glycine, as follows:



$$\begin{array}{l} CH_2Cl \\ | \\ CONHCH_2COOC_2H_5 \\ \text{chlor-acetyl glycine ester} \end{array} + H_2O = \begin{array}{l} CH_2Cl \\ | \\ CONHCH_2COOH \\ \text{chlor-acetyl glycine} \end{array} + C_2H_5OH$$

$$\begin{array}{l} CH_2Cl \\ | \\ CONHCH_2COOH \\ \text{chlor-acetyl glycine} \end{array} + NH_3 = \begin{array}{l} CH_2NH_2 \\ | \\ CONHCH_2COOH \\ \text{glycyl glycine} \end{array} + HCl$$

Instead of using chlor-acetyl chloride, we might have used alpha chlor-propionyl chloride $CH_3CHClCOCl$, in which case an alanyl group would have been introduced, giving alanyl glycine.

If the chlor-acyl amino acid compound is allowed to react with thionyl chloride the carboxyl group will be converted into an acid chloride group. The product can be treated with an amino acid ester, after which the ethyl group is removed and by the action of ammonia a tripeptide will be formed. The reactions are:

$$ClCH_2CONHCH_2COOH + SOCl_2 = ClCH_2CONHCH_2COCl$$

$$ClCH_2CONHCH_2COCl + H_2NCH_2COOC_2H_5 = ClCH_2CONHCH_2CONHCH_2COOC_2H_5$$

$$ClCH_2CONHCH_2CONHCH_2COOC_2H_5 + H_2O = ClCH_2CONHCH_2CONHCH_2COOH + C_2H_5OH$$

$$ClCH_2CONHCH_2CONHCH_2COOH + NH_3 = H_2NCH_2CONHCH_2CONHCH_2COOH$$

diglycyl glycine

By a continuation of this procedure the peptide chain can be lengthened at either end indefinitely. The most complex polypeptide synthetized by Fischer was leucyl-triglycyl-leucyl-triglycyl-leucyl-octaglycyl glycine, containing eighteen amino acids and having a molecular weight of 1213. While this compound is one of the most complex substances of known constitution that has ever been synthesized, it is a very simple substance as compared with the proteins,

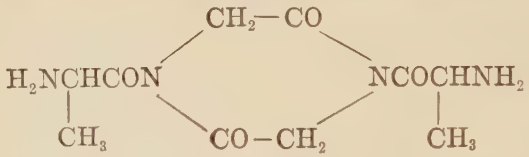
which are sometimes composed of twelve or fifteen different sorts of amino acids and may have molecular weights as high as from twenty to eighty thousand.

Polypeptide Reactions.—Polypeptides composed of three or more amino acids give the biuret test, the other color reactions are given if the requisite groups are present. The more complex polypeptides can be precipitated with tannic acid and phosphotungstic acid and can be salted out of solution. We can probably consider the polypeptides to be identical in structure with the peptones. The most convincing proof of this identity is the fact that trypsin and erepsin and enzymes in the tissues similar to erepsin are able to hydrolyse many of the polypeptides with the liberation of amino acids. The digestion of polypeptides has been found to be influenced by the length of the chain and by the configuration of the amino acid groups that are present. Thus, glycyl glycine was not hydrolysed, while diglycyl glycine was hydrolysed. Polypeptides containing unnatural amino acids like l. alanin and d. leucin were not attacked.

The Structure of the Protein Molecule

The protein molecule is believed to be similar in nature to the polypeptides. The $-CONH-$ groups serve to connect the amino acid radicals and are spoken of as the "protein ties." In addition to groups of this kind, proteins contain acid amide groups, $-CONH_2$, free amino groups, $-NH_2$, imide groups, $=NH$, and free carboxyl groups, $-COOH$. The polypeptide linkage does not explain all of the reactions of the proteins, such as heat coagulation and the difference between the action of pepsin and trypsin. There is considerable likelihood that the protein molecule may contain closed rings. Abderhalden believes that diketopiperazine rings are present. This belief is strengthened by the observation that small quantities of diketopipera-

zines, or amino acid anhydrides are found in protein hydrolysates; although it must be admitted that these anhydrides might be formed from the free amino acids by the action of boiling acid. An example of the diketopiperazine linkage suggested by Abderhalden is shown below. The compound is composed of two molecules of glycine and two of alanin:



The solution of the problem of the exact structure of proteins is as important as it is difficult. Until considerable progress has been made in this direction it is not likely that we shall be able to understand the true nature of enzyme action, or the reactions of immuno chemistry.

PHYSICAL AND CHEMICAL DIFFERENCES AMONG PROTEINS

While the proteins can be considered as anhydrides of the amino acids, they exhibit among themselves great differences in chemical and physical properties. These differences are doubtless due in part to the nature of the amino acids present and in part to the chemical structure. The insolubility of the keratins may depend upon the large amount of cystin present; the properties of gelatin may be connected with the high percentage of glycocoll. Fur and feathers (keratin) are of value on account of their indestructibility and their insulating air spaces. Ligaments (collagen) are remarkable for their strength, horns and claws (keratin) for their toughness. The protein in saliva (mucin) is an effective lubricant, while that in the arterial walls (elastin) is elastic and aids greatly in the circulation of the blood. The proteins in the crystalline lens of the eye serve to refract light. The fibrinogen of the blood is especially designed to stop leaks. The proteins of milk

(casein and lactalbumin) have been prepared as food for the young mammal, those in egg yolk (ovovitellin) are to serve as food for the young bird.

It seems probable that the cell uses protein, or the amino acids from protein, as material from which to construct hormones, enzymes and antitoxins. Hereditary differences probably depend upon the immeasurably complex structure of the protein molecule.

Protein Digestion

In Liebig's time it was held that the object of protein digestion was to bring proteins into solution that they might be absorbed without further alteration. At the present time it is known that proteins must not only be made soluble, but that they must also be denatured. When foreign proteins are admitted into the circulation they are likely to bring about the reaction of anaphylaxis and proteoses and peptones are poisonous if injected intravenously.

Gastric Digestion

The gastric juice secreted by the mucous coat of the stomach contains the enzymes pepsin and rennin, which are produced by the chief or central cells, and hydrochloric acid, which is produced by the parietal cells. The central cells are found both in the prepyloric and pyloric regions of the stomach, while the parietal cells are limited to the prepyloric part. The concentration of hydrochloric acid in human gastric juice is nearly twentieth normal. During gastric digestion proteins swell up and dissolve, being hydrolysed to proteoses and peptones. Amino acids are formed only to a slight extent. Among the proteins not attacked by gastric juice are mucin, reticulin and the keratins. Although proteins combine with the hydrochloric acid to form salts, free hydrochloric acid is always present

during gastric digestion. The amount of free hydrochloric acid in the gastric contents, as well as the total acidity (free and combined), shows a time curve which is characteristic. Under pathological conditions there may be a hypo- or a hyper-secretion of hydrochloric acid. Again, in abnormal cases the emptying of the stomach may be retarded.

This indicates the necessity for repeated examination of gastric contents at short intervals. A single examination one hour after a test meal, for example, might give the same values for very different conditions.

When hydrochloric acid is diminished, or absent, from the gastric juice, fermentation processes go on, with the production of lactic acid, and sometimes of butyric acid. Free hydrochloric acid can be tested for by the use of Congo red paper, or better, by the Günzberg reagent. Lactic acid is tested for by adding ferric chloride to an ethereal extract of the gastric contents. In the presence of lactic acid an intense yellow color is produced. It is seldom necessary to examine gastric contents for the presence of pepsin as pepsin is practically never absent, unless hydrochloric acid is absent too.

Rennin (rennet, or chymosin) is a proteolytic enzyme found in gastric juice. The only action ascribed to rennin is the conversion of casein into paracasein which is necessary for the curdling of milk. Rennin is said to act best in a faintly acid solution and in the presence of calcium ions.

Intestinal Digestion

When the acid chyme from the stomach enters the duodenum it is neutralized by the sodium bicarbonate from the succus entericus, pancreatic juice and bile. The pancreatic juice supplies the proteolytic enzyme *trypsin,* which brings about a further hydrolysis of protein materials with the formation of peptides and amino acids. Trypsin acts most efficiently in nearly neutral solutions,

but is capable of action when the reaction is either faintly acid or faintly alkaline. The optimum temperature for trypsin is body temperature. Trypsin is a more efficient catalyst in vivo than in vitro, the reason probably being that in the animal the products of hydrolysis are rapidly removed by diffusion through the intestinal wall, or are attacked by the enzyme erepsin. The pancreatic juice has a rennet action on milk but it is possible that this is due to trypsin, inasmuch as all proteolytic enzymes have this effect.

In the intestinal mucosa and in the succus entericus *erepsin* is present. This enzyme concludes the work initiated by pepsin and continued by trypsin. With the exception of fibrin and casein, erepsin does not digest unaltered proteins, having the function of splitting proteoses and peptones to amino acids. Since erepsin is present in the intestinal mucosa, it seems unlikely that peptones can be absorbed before being hydrolysed. Enzymes similar to erepsin in many respects are found in most animal and plant tissues. Unlike intestinal erepsin the tissue erepsins of animals are usually most active in acid media and are responsible for the *autolysis,* or self-digestion of the tissues that occurs after death. Autolysis has been studied by Jacoby and especially by Bradley and his collaborators.

From the foregoing, it will be seen that the three digestive enzymes, pepsin, trypsin, and erepsin are supplementary in their action; trypsin begins where pepsin leaves off, and erepsin concludes the hydrolysis. Inasmuch as the products of digestion are absorbed as rapidly as they are formed, the hydrolysis of proteins in the gastro-intestinal tract is both rapid and efficient.

The digestion and absorption of ingested protein is usually very complete. When certain vegetable proteins are fed, the availability may be considerably less, on account of the presence of cellulose, which both prevents the diges-

tive juices from coming in contact with the protein and also increases peristalsis by mechanical irritation.

The amount of nitrogen in the feces, which in man normally is found to be about 1 gm. for every twenty-four hours, cannot be considered to come entirely from unabsorbed food protein, but is largely due to intestinal secretions, cell detritus, and bacterial cells.

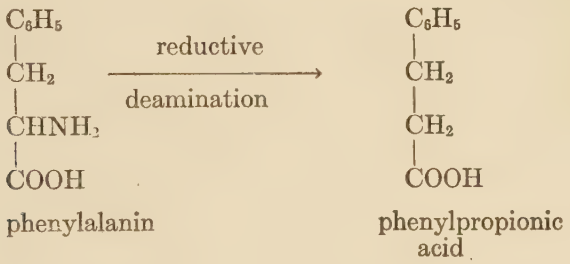
Intestinal Putrefaction.—The action of bacteria on proteins and protein decomposition products is called putrefaction. Under normal conditions the amount of putrefaction is small. It takes place chiefly in the large intestine. The sterilizing action of the hydrochloric acid of the gastric juice, the inhibiting action of fermentative processes, and the rapid digestion and absorption of food materials prevent intestinal putrefaction. Whenever there is a lack of hydrochloric acid in the gastric juice, or whenever there is a stasis of material on account of constipation, putrefactive processes are increased. The products formed from proteins by the action of bateria are, in the first place, proteoses, peptones, and amino acids, but bacterial action proceeds farther and decomposes amino acids with the formation of such substances as indol, skatol, phenol, cresols, ammonia, hydrogen sulphide, carbon dioxide, fatty acids, and amines (putrescin, cadaverin, histamin). It must be remembered that bacteria are capable of attacking not only the food proteins, but also those present in the digestive juices.

The harmful effect of bacterial putrefaction is caused by the absorption and action upon the body of certain products. Headaches, chills, fever, and a depressing action on the heart are some of the symptoms. Of the various medicines given to prevent protein putrefaction, one of the best is calomel, which is efficient on account of its cathartic effect. The bacillus acidophilus is thought to be of value in the treatment of intestinal putrefaction because its

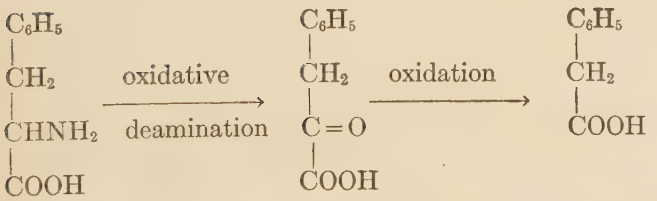
growth is antagonistic to that of the harmful bacteria. Diets containing considerable quantities of lactose are said to favor the growth of bacillus acidophilus in the intestine.

Alvarez has pointed out that we have little positive evidence that the products of intestinal putrefaction are the cause of the symptoms of indigestion. He believes rather that the symptoms are caused by a distension of the colon and by antiperistaltic contractions of the digestive tract. He considers that the more serious consequences of auto-intoxication are due to the passage of living bacteria from the intestine into the blood stream.

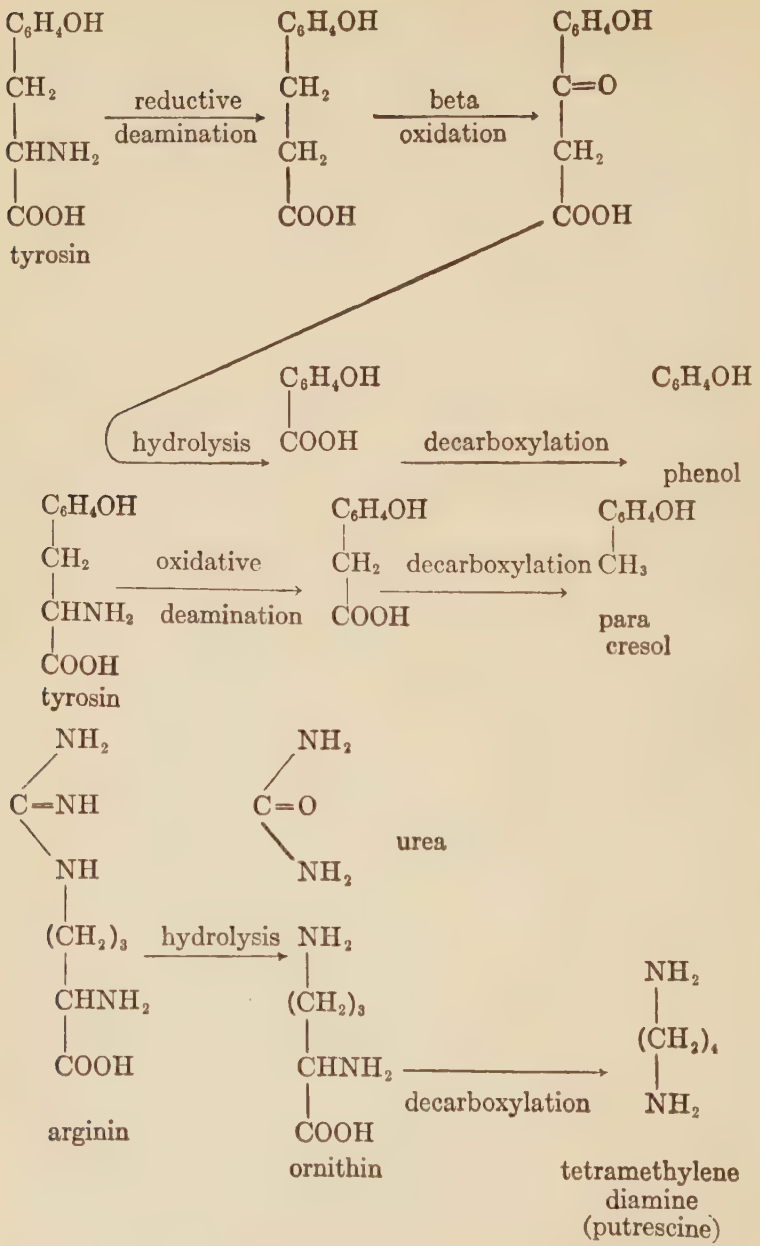
The following reactions are typical examples of the action of putrefactive bacteria upon amino acids. It will be seen that these reactions involve oxidative and reductive deamination, beta oxidation and decarboxylation,—resulting in the formation of fatty acids, amines, phenols, etc.

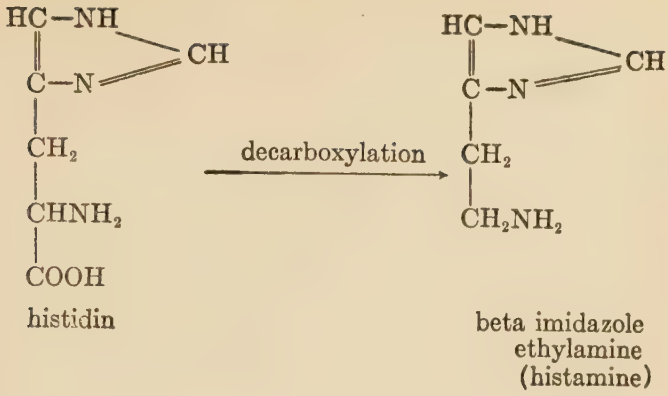


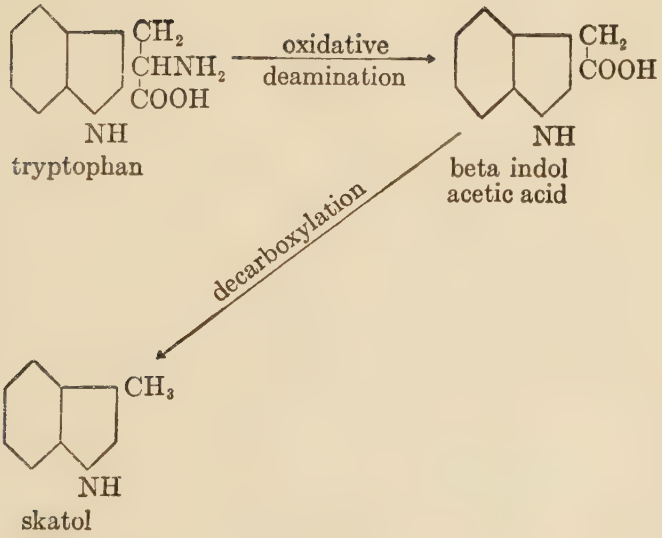
phenylalanin — phenylpropionic acid

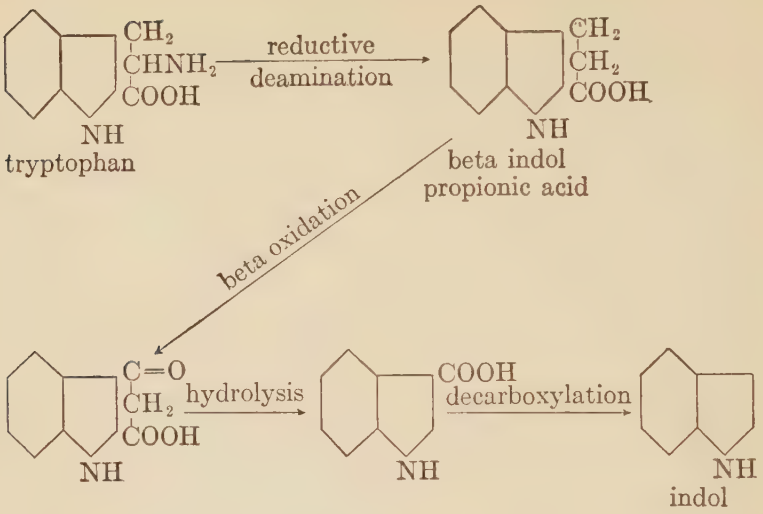


phenylalanin — phenylpyruvic acid — phenylacetic acid


C6H4OH
CH2
CHNH2
COOH
tyrosin
reductive deamination
C6H4OH
CH2
CH2
COOH
beta oxidation
C6H4OH
C=O
CH2
COOH
hydrolysis
C6H4OH
COOH
decarboxylation
C6H4OH
phenol
C6H4OH
CH2
CHNH2
COOH
tyrosin
oxidative deamination
C6H4OH
CH2
COOH
decarboxylation
C6H4OH
CH3
para cresol
NH2
C=NH
NH
(CH2)3
CHNH2
COOH
arginin
hydrolysis
NH2
C=O
NH2
urea
NH2
(CH2)3
CHNH2
COOH
ornithin
decarboxylation
NH2
(CH2)4
NH2
tetramethylene diamine (putrescine)


HC—NH
CH
C—N
CH2
CHNH2
COOH
histidin
decarboxylation
HC—NH
CH
C—N
CH2
CH2NH2
beta imidazole
ethylamine
(histamine)


CH2
CHNH2
COOH
NH
tryptophan
oxidative
deamination
CH2
COOH
NH
beta indol
acetic acid
decarboxylation
CH3
NH
skatol



Protein Absorption

Early physiologists believed that proteins were digested only to the peptone stage, and tried to find peptones in blood. As they were unable to do this, they brought forward the hypothesis that peptones were resynthesized by the intestinal epithelium to proteins, which were indistinguishable from blood proteins. When erepsin was discovered by Cohnheim, it was seen that protein digestion continues to the amino acid stage. It was thought that amino acids should be present in the blood during protein digestion, but no amino acids were found by early investigators.

At this time, the protein resynthesis theory was revived by Abderhalden. Another theory brought forward attempted to explain the absence of amino acids in the blood as due to their immediate deaminization while passing through the intestine.

In 1906, Howell dialysed dog's blood and attempted to precipitate amino acids from the dialysate with napthyl sulpho-chloride. He obtained an oil.

In 1912, Folin and Denis demonstrated that amino acids are transported by the blood to the tissues. They introduced solutions of amino acids into isolated loops of the intestines of cats. At the end of the experiment the unabsorbed material was washed out and estimated. The difference represented the amount of amino acid that had been absorbed. Analyses of the blood were made from time to time to trace the absorption of amino acids. Folin and Denis did not attempt to isolate the amino acids from blood, but determined the non-protein nitrogen (N. P. N.) by precipitating the blood proteins and running micro-Kjeldahl determinations upon the filtrates. The non-protein nitrogen of blood is contained in such compounds as urea, creatin, amino acids, uric acid, and creatinin. The table below gives the results of one of these experiments.

TABLE

Glycocoll N introduced	1867 mgs.
Glycocoll N recovered	1300 mgs.
Glycocoll N absorbed	567 mgs.

	Mgs. N. P. N. per 100 cc. of blood
Initial N. P. N., portal blood	30
After 6 min., portal blood	36
After 6 min., carotid blood	36
After 18 min., portal blood	55
After 18 min., carotid blood	47
After 45 min., mesenteric blood	85
After 45 min., carotid blood	57

The initial N. P. N. in muscle was 250 mgs. and the final N. P. N. 346 mgs. per 100 gms.

Later Van Slyke and Meyer traced the absorption of amino acids in the dog, using Van Slyke's method for the gasometric estimation of amino nitrogen in the protein-free blood filtrates. They found that a considerable amount of amino acids was absorbed by the liver, but that other

tissues also participated in the absorption. The amino acid nitrogen content of the blood was found to increase from 4 mg. per 100 cc. at the start to 6 mg. during the absorption.

Abel, using his method of vividiffusion by means of celloidin tubes, has been able to isolate alanin from the blood; and Abderhalden, by using large quantities of blood, has succeeded in isolating ten different amino acids. To-day there is no question that amino acids pass from the intestine into the mesenteric veins and that they are conveyed to all parts of the body. The concentration of amino acids in the blood does not increase greatly during absorption from the intestine because they are rapidly removed by the tissues. The blood stream does not serve as a reservoir for amino acids, but merely as a means of transportation.

Protein Metabolism

The amino acids that are absorbed by the tissues are used to a certain extent for the reconstruction of protoplasm, but for the most part they are rapidly deaminized with the formation of urea. From 6 to 8 hours after the ingestion of protein, urea formation is at a maximum. The non-nitrogenous portion of certain of the amino acids is converted into glucose. In the diabetic animal the glucose that is formed in this manner is eliminated in the urine more rapidly than the urea.

Nitrogen Equilibrium.—Carl von Voit studied the nitrogen metabolism in dogs and found that except for a small amount of nitrogen in the feces, nearly all of the nitrogen is eliminated by way of the urine. Von Voit kept a balance sheet showing the amount of nitrogen ingested and excreted by the dogs over long periods of time. He found that if the diet were kept constant the nitrogen elimination always became equal to the nitrogen intake within two or three days. In other words, the animal arrived at a state

of nitrogen equilibrium. The phenomenon of nitrogen equilibrium is a proof of the inability to store nitrogen.

An animal may be in a state of nitrogen equilibrium at a high level, or at a low level, depending upon the amount of protein food fed. Growing animals and those recovering from wasting diseases are naturally not in a state of nitrogen equilibrium. When a patient is suffering from a disease such as typhoid fever, nitrogen equilibrium cannot be obtained, no matter how much protein is present in the diet.

Protein Catabolism.—The amino acids that are absorbed from the intestine and transported to the cells are used to a slight extent for the repair of protoplasm, or for growth. The balance is rapidly deaminized, the nitrogen going to form urea and the remainder of the molecule being converted into glucose, or else being oxidized without conversion to glucose. We can consider that most of the amino acids travel a short route, inasmuch as they are catabolized immediately. The amino acids that are built into tissues traverse a long route, but are ultimately split off and catabolized. It is impossible to tell how long living protoplasm remains intact, and it is difficult to understand why it is being constantly catabolized and rebuilt, but such appears to be the case. It is possible to hasten the destruction of the body protein by starvation, or by giving such poisons as arsenic, or phosphorus. It is also possible to decrease the destruction of the body protein by giving sufficient food, but the catabolic process cannot be prevented entirely.

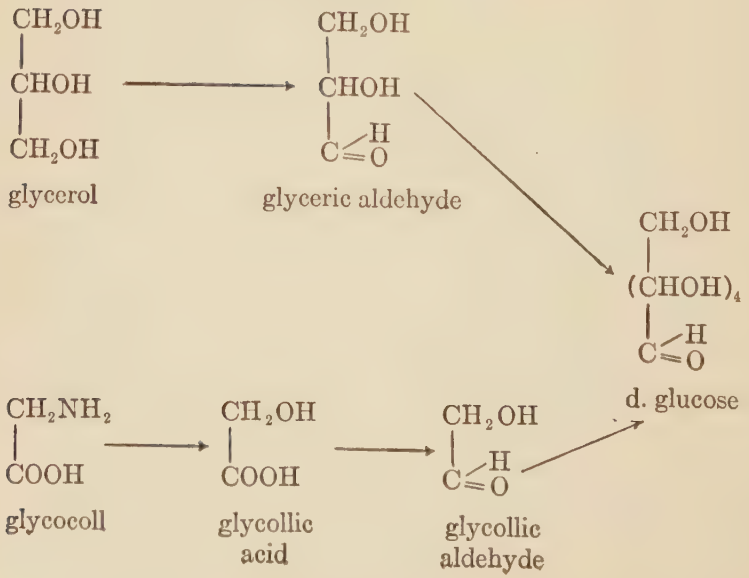
The end-products of protein catabolism are urea, carbon dioxide, water, sulphuric acid and phosphoric acid. The rate of protein catabolism is most readily measured by estimating the excretion of urea. Since the average protein contains 17 per cent of nitrogen it is possible to obtain the figure for catabolized protein by multiplying urea nitrogen by the factor 6.25.

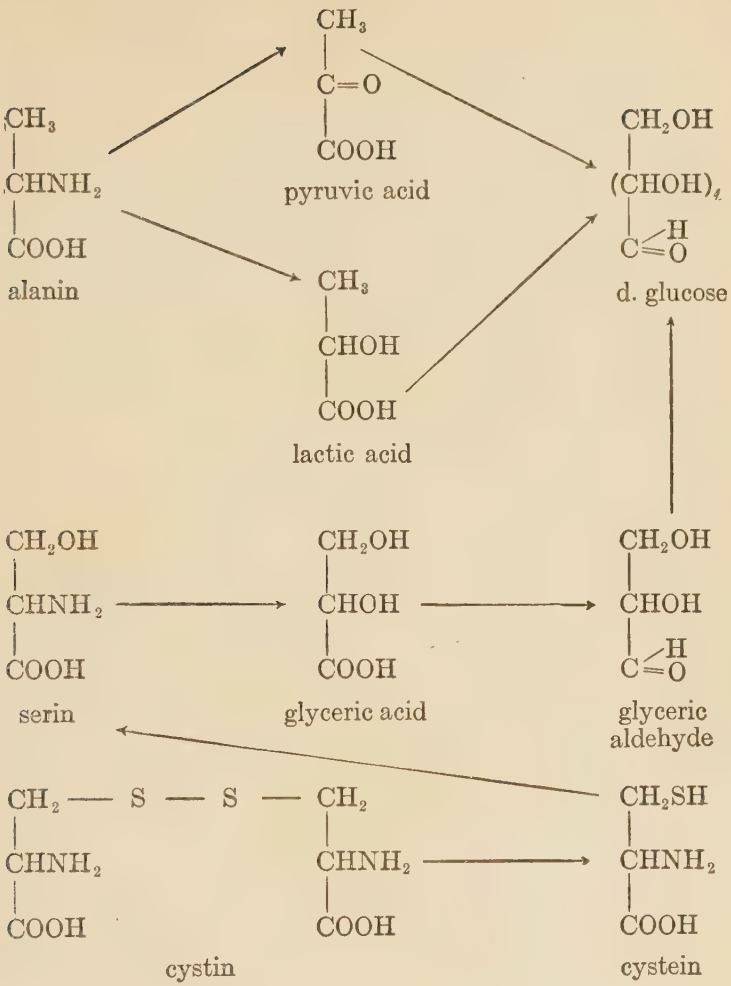
The Formation of Glucose

We have seen already that glucose is formed by the liver from the monosaccharides fructose, galactose and mannose (see page 59). Below are indicated some of the reactions that are possibly involved in the synthesis of glucose from glycerol and from some of the amino acids.

The following amino acids have been proved to be converted into glucose by the animal. The procedure consisted in feeding the amino acid in question to a totally diabetic dog and estimating the extra glucose eliminated in the urine.

glycocoll
alanin
prolin
aspartic acid
glutamic acid
serin
cystin
arginin

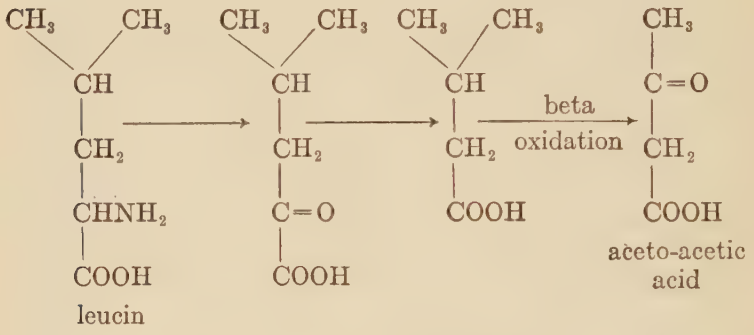




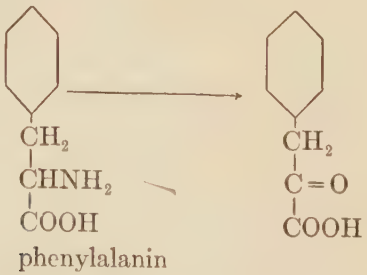
It has been shown that glycocoll contributes 2 carbon atoms to the formation of glucose. Alanin, prolin, aspartic acid, glutamic acid, serin and arginin each contributes 3 carbon atoms and cystin contributes 6 carbon atoms.

The amino acids leucin, tyrosin and phenylalanin do not give rise to glucose, but when fed to a diabetic animal

are converted into aceto-acetic acid. The production of this acid from leucin can probably be represented by the following reactions:

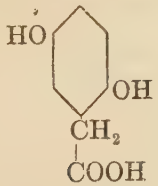


It is of interest to note that while the animal is incapable of oxidizing such compounds as benzoic acid and phenyl acetic acid and is usually unable to rupture the benzene ring, the amino acids phenylalanin and tyrosin are very readily and completely oxidized. According to Dakin, the ease with which these two amino acids are catabolized is dependent upon the structure of the side chain. The first step in the process of catabolism is deaminization, with the production of a keto acid:

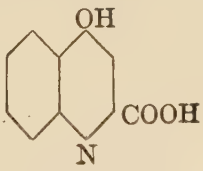


The next reaction probably involves the entrance of oxygen into the benzene ring with the ultimate rupture of the ring. The exact manner in which aceto-acetic acid is pro-

duced is not known. Some light has been thrown on the catabolism of phenylalanin and tyrosin by investigation of the abnormal metabolic condition that is known as alkaptonuria. In this rare disease phenylalanin and tyrosin are not completely oxidized and give rise to homogentisic acid, which appears in the urine. Homogentisic acid is readily oxidized by atmospheric oxygen, and urine containing this substance becomes black upon standing. It appears likely that homogentisic acid is an intermediate product in the normal catabolism of both phenyl alanin and tyrosin, and that in the alkaptonuric person the process has stopped at this point. It is rather remarkable that the formation of homogentisic acid from tyrosin involves the shifting of the hydroxyl from the para position.



homogentisic acid



kynurenic acid

Little is known about the intermediary metabolism of the other amino acids. In dogs tryptophan is in part converted into the substance known as kynurenic acid, which is eliminated in the urine. This reaction is interesting and unusual in that the pyrrol ring of the tryptophan is changed into a pyridine ring.

It should be mentioned that the animal probably uses the amino acids tyrosin and phenylalanin for the synthesis of the hormones adrenalin and thyroxin (see page 128).

Protein Metabolism in Starvation

On the first day of starvation the animal lives on the glycogen stored in its liver. On the second day of starvation the store of glycogen has been largely used up and a

greater amount of protein is catabolized in order to produce energy. During the period of starvation about 15 per cent of the energy is derived from protein and about 85 per cent from fat. The liver never becomes entirely free from glycogen as some is formed from the protein that is catabolized. During the first 6 days of starvation the nitrogen

adrenalin

thyroxin
(Harington formula)

elimination is high if the animal has been previously well fed. What is known as deposit protein is being destroyed. After prolonged fasting the nitrogen excretion in man has been known to sink to about 3 grams daily. This is equivalent to a daily destruction of between 18 and 20 grams of protein. The production of acetone bodies during starvation is considerable but is not dangerous. When the available fat of the body has been used up, the destruction of tissue protein increases and larger amounts of nitrogen are excreted. This is known as the pre-mortal rise of nitrogen and is a sign that death is near.

The longest record for a fast with subsequent recovery in man is 50 days, for a dog 127 days.

Protein Nutrition

While fats and carbohydrates are of value to the animal for the production of energy and heat, the proteins are valuable not only for the energy that they yield, but more especially for the construction of protoplasm. The amino acids that are absorbed during digestion are used by the growing animal for construction and by the adult for repair.

The Protein Requirement.—Carl von Voit believed that in a starving animal protein destruction would be at its lowest level. He expected that if he fed the animal an amount of protein equivalent to the amount destroyed daily during starvation that nitrogen equilibrium would result, but this was found not to be so. For every part of the animal's own flesh destroyed during starvation von Voit was obliged to feed about five parts of meat in order to obtain nitrogen equilibrium.

Fourteen years later similar experiments were carried out by E. Voit and Korkunoff. These experimenters found that dogs required three and one-half times as much protein in the diet for the maintenance of nitrogen equilibrium as was broken down during starvation. However, when an ample amount of carbohydrate was fed together with the protein the amount of protein necessary for nitrogen equilibrium was about the same as the amount destroyed during starvation. When fat was fed in place of the carbohydrate the amount of protein that had to be fed in order to maintain nitrogen equilibrium was 1.6 to 2.1 times the amount destroyed daily during starvation. These results are important because they show the sparing action of both fat and carbohydrate on the metabolism of protein, carbohydrate being the more efficient of the two. The sparing action is due to the fact that fat and carbohydrate can be used by the animal for the production of energy in place of protein. When a sufficient amount of carbohydrate is at hand tissue protein will not be destroyed and the endogenous metab-

olism of protein will be at a minimum. Under these circumstances the daily destruction of protein in man is about 20 grams.

Below are given standard diets for light work and for hard work that were compiled from statistical evidence by C. von Voit. It will be seen that more protein and fat is consumed by the person doing hard work, while the amount of carbohydrate remains the same. From what we know of the sparing action of carbohydrate upon protein breakdown it seems evident that the diet for hard work should contain above all an increased amount of carbohydrate; an increase in the amount of fat would be desirable also, but the quantity of protein should remain the same, for reasons to be discussed later.

STANDARD DIETS OF C. VON VOIT

	Fat	Protein	Carbohydrate
	gm.	*gm.*	*gm.*
Light work.....	56	118	500
Hard work.....	100	145	500

The question of the proper amount of protein for the human diet has never been settled. Advocates of a low-protein diet believe that the use of large quantities of protein is not only economically wasteful, since proteins are more expensive than are carbohydrates or fats, but that it is even detrimental to health. Their chief argument is based upon the fact that the animal is unable to store protein above the small amount used for reconstruction and for growth, but is obliged to deaminize the surplus of amino acids and eliminate the nitrogen as urea nitrogen. Some advocates of the low protein diet believe that the production and elimination of urea in large quantities put

a strain on the body. They also point out that diets high in protein are likely to give rise to intestinal putrefaction and that the specific dynamic action of protein, which is so much greater than that of either carbohydrate or fat, makes the consumption of large quantities of protein undesirable for persons living in a hot climate.

Advocates of the high protein diet like to point out that the races of mankind that are accustomed to consume large amounts of protein are the most vigorous and the most successful, while the races that consume small quantities of protein are indolent and sluggish. Moreover, advocates of the high protein diet believe that a surplus of protein is necessary to assure resistance to disease.

It has never been proved that a high protein diet is harmful, but it seems likely that a low protein diet is better suited to those living in the tropics on account of the heating effect of protein food. This is attested to by the fact that inhabitants of hot climates are nearly always found to prefer a low protein diet even when protein is readily to be obtained. Owing to the deficiency of certain proteins in amino acids, it is always possible that a diet that is especially low in protein may be close to the danger line, unless the food that is chosen contains protein of the highest biological value.

Feeding Experiments with Amino Acids.—The question of the extent of protein digestion as well as that of the comparative food value of different proteins, has led a number of investigators to feed animals with protein digests and mixtures of amino acids. If an adult animal is capable of nitrogen equilibrium, or if a growing animal is capable of nitrogen retention when given a diet of simple amino acids together with such amounts of fat and carbohydrate as may be found necessary, we may be led to believe that no more complex substances than amino acids are absorbed from the intestinal tract.

Loewi fed dogs autolysed pancreas, which was nearly abiuret, together with fat and carbohydrate. He found nitrogen equilibrium and nitrogen retention in some cases. Henderson and Dean hydrolysed meat with sulphuric acid and fed the material to dogs. They found nitrogen equilibrium. Abderhalden and Rona worked with mice. They found that acid hydrolysis destroyed something present in casein which was necessary for the nutrition of the mice. This necessary constituent was found to be the amino acid tryptophan. Henriques and Hansen found that rats fed on casein hydrolysed by acid died, but obtained nitrogen equilibrium when they fed the rates on ox pancreas which had been autolysed and then digested by erepsin.

Abderhalden fed a dog a mixture of 17 different amino acids and found nitrogen retention. Thus, it appears that it is the amino acids in protein and not more complex substances that are necessary for nutrition.

It must be said in closing that we have no proof that certain of the simpler peptides are not absorbed from the intestine. It is known that some of the dipeptides, such as glycyl glycine, are not hydrolysed by trypsin and erepsin. It has been observed also that traces of peptide nitrogen are present in the blood.

The Biological Values of Proteins

The biological values of the proteins depend upon their use to the animal for growth, maintenance, reproduction and lactation. In the process known as "maintenance" a certain amount of protein is doubtless used up for the elaboration of enzymes, especially those of the digestive tract, and for the synthesis of hormones, such as thyroxin and adrenalin. It is also possible that the maintenance requirement of animals is due to the necessity of replacing cells that have died. It has been seen that the destruction of body protein during starvation sinks to a low value and

that a low value is also obtained when animals are fed on non-protein diets containing sufficient carbohydrate and fat to supply all of the requirements for energy. Whether or not this endogenous destruction of body protein still continues when the animal is being fed on a diet containing an ample supply of sufficient proteins is a point that has never been satisfactorily answered, as it is impossible to distinguish between the endogenous and the exogenous nitrogen of the urine when both are present at the same time. The fact that the nitrogen metabolism is decreased by feeding substances such as urea and ammonium salts of organic acids, would lead one to think of the protein catabolism as a balanced reaction. However, it may not be possible to stop this reaction completely.

The biological value of proteins might be expected to depend entirely upon the kind and amount of the various amino acids present, provided the digestion were complete. This theory has been largely borne out by feeding experiments, although in some cases the evidence is conflicting, or to the contrary. It appears that the requirements for growth are different from those for maintenance, and we might expect that the kind of amino acids required for lactation and for reproduction would be still somewhat different. From analytical values obtained for the amino acid content of proteins it is evident that certain proteins contain neither a sufficient assortment of amino acids nor a desirable percentage thereof. Gelatin contains no tryptophan and not more than traces of tyrosin and cystin. Gliadin of wheat contains no glycocoll or lysin, and nearly 40 per cent of the molecule is composed of glutamic acid. Zein of corn (maize) lacks glycocoll, tryptophan and lysin.

It has been found that gelatin, when fed together with carbohydrate, fat and mineral salts, is not capable of supporting life. Zein from corn has been shown by Osborne and Mendel to be another insufficient protein. When the

amino acids tryptophan, tyrosin, and cystin are fed together with gelatin, life can be maintained. Gliadin has been found capable of maintenance but is not sufficient for growth. Certain amino acids are present in this protein in amounts sufficient only for maintenance. Casein and lactalbumin have been found sufficient in themselves for both maintenance and growth. When the diet contained only 9 per cent of casein, however, Osborne and Mendel found that rats could not grow unless cystin was added. With a diet containing 9 per cent of lactalbumin rats could grow. Among vegetable proteins some of those sufficient in themselves for growth are: glutenin of wheat, edestin of hemp, excelsin of the Brazil nut, the glutelin of corn, and many others.

Thomas-Mitchell Method.—The nutritive values of proteins can be determined by feeding food that contains as the source of nitrogen only the protein in question, and then observing the ability of the animal to grow, to reproduce and to nurse its young. Another method consists in determining the nitrogen balance.

Mitchell has modified the Thomas method for determining the biological values of proteins. As this method is particularly instructive it will be explained in detail.

Rats were used as experimental animals. The diet contained enough fat and carbohydrate to assure a plentiful supply of energy so that the protein would not be used primarily for the production of energy. The diet also contained minerals and the vitamins A and B. The amount of food consumed by the rats was carefully measured. The wastage of food during digestion was found to depend upon the amount of food eaten and upon the amount of roughage present. In order to determine what proportion of fecal nitrogen corresponded to the intestinal secretions and cell detritus, the rats were first fed on a protein-free diet and the value for fecal nitrogen per gram of food was deter-

mined. As the amount of roughage in the food mixture was constant this factor did not influence the results. When protein was added to the diet the extra nitrogen in the feces then could be taken to represent the unabsorbed food protein. The difference between the protein ingested and the protein that was not absorbed gave the amount of protein that was absorbed.

The determination of the wastage of food protein in metabolism depended on the assumption that there is a certain minimum endogenous protein catabolism that cannot be diminished by the diet. This endogenous protein catabolism was measured by placing the rats on protein-free food for several days and measuring the total nitrogen in the urine. By multiplying the daily urinary nitrogen elimination by the factor 6.25 the minimal daily destruction of endogenous protein was obtained. The rats were then fed on a diet containing the protein, or mixture of proteins, which it was desired to test. The diet usually contained 5 per cent of protein since it has been found that the economy with which an animal uses its food protein depends upon the amount present and that the differences in the biological values become less marked at higher levels. The amount of nitrogen eliminated in the urine was considered to represent both the unavoidable tissue metabolism and the wastage of food protein. By subtracting the value for the former the nitrogen from the wastage of food protein was obtained. This value could now be subtracted from the value of the food protein absorbed, the difference being the nitrogen stored in the tissues. The percentage of the absorbed nitrogen that was stored in the tissues was expressed as the biological value of the protein or proteins used in the experiment.

In order to make clear this method of determining the biological value of proteins an example is taken from an article by Mitchell. The rat was fed on a ration containing

approximately 4 per cent of a mixture of proteins derived from corn and tankage—a packing-house product. Mitchell reports: "The rat consumed an average of 56.9 mg. of nitrogen per day in a 7-day collection period. On this diet the daily excretion of fecal nitrogen was 27.6 mg., but of this nitrogen it is estimated by the aid of the fecal nitrogen excreted on a nitrogen-free diet, that 21.7 mg. were derived from the body, leaving only 5.9 mg. contained in the indigestible food protein. Hence the rat was receiving daily 56.9−5.9=51.0 mg. of absorbed nitrogen. During this period the excretion of urinary nitrogen was 48.6 mg. daily, of which 37.7 mg. were the result of the catabolism of the body's own tissues, estimated from the excretion of urinary nitrogen on a nitrogen-free diet. Hence only 48.6−37.7= 10.9 mg. of the absorbed dietary nitrogen were wasted in metabolism, and 51.0−10.9=40.1 mg. were retained in the body. The biological value of the protein, therefore, is equal to $100\times(40.1\div51.0)=79$."

In the following table are given some of Mitchell's results for the biological values of proteins, or mixture of proteins,

TABLE SHOWING THE BIOLOGICAL VALUES OF 5 PER CENT AND 10 PER CENT PROTEIN DIETS, AFTER MITCHELL

Material fed as a source of Nitrogen	Biological Values	
	5% level	10% level
casein	70.8	.....
milk proteins	93.4	84.7
corn proteins	72	59.6
oat proteins	78.6	64.9
rice proteins	86.1	.....
navy beans proteins	.....	38.4
potato nitrogen	68.5	66.7
yeast nitrogen	85.5	.....
tankage	.....	31.5

with diets containing 5 per cent of protein, and with diets containing 10 per cent of protein. Since about 40 per cent of the nitrogen in the potato is not protein nitrogen but consists of the nitrogen of amino acids and of amino acid derivatives, the term "potato nitrogen" has been used to denote a diet containing this material. The same thing applies also to a diet containing yeast.

Can the Animal Synthesize the Amino Acids?

Since animal life cannot be maintained when such inadequate proteins as gelatin or zein are the sole source of protein food, we are led to believe that unlike plants, animals are incapable of producing synthetically certain of the amino acids. It has been well established that animals are incapable of synthesizing the benzol ring which is necessary for the production of phenylalanin, tyrosin and tryptophan. It has been proved that tyrosin, tryptophan, lysin and cystin cannot be synthesized by the animal.

Only two amino acids have so far been shown to be capable of synthesis by animals; these are glycocoll and glutamic acid. Magnus Levy fed large quantities of benzoic acid to rabbits. The hippuric acid excreted in the urine contained more glycocoll than could have existed preformed in the rabbits' tissues, and must have been formed synthetically. It has been shown that in this synthesis urea furnishes the nitrogen. When benzoic acid is fed to man it is converted into hippuric acid and also glutamuric acid. Sherwin believes the glutamic acid used for the production of glutamuric acid to be formed synthetically. In birds the feeding of benzoic acid results in the production of ornithuric acid, indicating a synthesis of ornithin, which though not a primary amino acid, is closely related to arginine. It has been found that no new amino acids were produced during the incubation of hen's eggs.

Embden and Schmitz have perfused surviving livers with ammonium salts of alpha-keto-acids and have found that amino acids are formed from these keto-acids. This reaction is not a true synthesis but merely the reverse of deaminization.

Chapter V

VITAMINS

Introductory

It has been seen that proteins are all-important for the purpose of nutrition, inasmuch as they are necessary for growth and for the repair of protoplasm. Carbohydrates and fats are valuable as producers of energy and incidentally of heat. In addition, water and inorganic salts must be supplied in order to replace the amounts that are continually being lost in the excretions. Until recently it has been believed that the above mentioned substances furnish all food requirements.

In 1899 Eijkman noted that the disease beriberi is caused by a one-sided diet of polished rice and that when unpolished rice is fed the disease does not develop. Röhmann found that mice did not thrive when fed highly purified food and that their young did not live. In 1906 Hopkins made the statement that some unknown substances in the diet are necessary for life. Funk has named these unknown substances vitamins, owing to an erroneous impression that the substance necessary for the prevention of beriberi was an amine. Mendel and Osborne, McCollum and Davis and many other investigators have carried out a multitude of feeding experiments on animals in order to ascertain the occurrence and distribution of vitamins in foods and the effects of diets deficient in vitamins. A considerable amount of work has been done in attempting to concentrate and isolate vitamins. As the work has progressed the importance and number of vitamins has grown. We know at the present time that at least five different vitamins

exist and that the continuation of animal life is impossible in the absence of any one of the five. It is impossible to say whether any more vitamins will be discovered in the future. In spite of the work already done, our knowledge of the chemical nature of the vitamins is practically nil. Nevertheless, it is reasonable to expect that the chemist will succeed in isolating these compounds and in explaining their chemistry at no distant date. This should not prove an impossible feat as the vitamins are not of colloidal nature and are not especially unstable.

Vitamin "A."—In the absence of specific knowledge of their chemical composition it has been necessary to give general names to the vitamins. The letters of the alphabet have been employed for this purpose. Vitamin A occurs in animal tissues in association with fat and is soluble in the fat solvents. For this reason this vitamin is sometimes spoken of as "fat-soluble A." In the absence of vitamin A, growth ceases and the eye disease, xerophthalmia, develops. Full-grown animals are less sensitive to the lack of vitamin A than growing animals; possibly this is on account of the animal's ability to store this vitamin in the body. Vitamin A is found in the liver and kidneys; small amounts are present in muscle. It is present in milk, in part associated with the fat, and is therefore found in butter. It is present in egg-yolk and cod liver oil. Small amounts are found in beef fat, but little or none in lard. Green leaves contain considerable amounts of vitamin A in spite of the fact that they are practically free from fat. In plants vitamin A is usually associated with carotin.

Vitamin A is resistant to saponification and remains in the unsaponifyable fraction after hydrolysing a fat with cold alkali. Drummond has distilled vitamin A in vacuo without destroying it. At high temperatures vitamin A is destroyed by oxidation, but it is unlikely that there is much loss of vitamin A in the cooking of foods.

Vitamin "B."—Vitamin B is water-soluble and is usually spoken of as water-soluble B. It is also soluble in dilute alcohol, but is not soluble in strong alcohol. When vitamin B is not present in the diet, animals lose weight and appetite. There is an increased susceptibility to bacterial infection. Lack of vitamin B leads to a condition known as polyneuritis in birds and beriberi in man. This disease is characterized by a general inflammation of the nerves, hypertrophy of the adrenals and paralytic symptoms. Water-soluble B is found in the pericarp of rice and other grains; it is present in nearly all parts of plants in considerable amount. Yeast contains large amounts. It is present in eggs and milk and in the livers of animals. Animals are unable to store vitamin B to any considerable extent. Since vitamin B is soluble in water, it is likely to be lost in part if the water in which vegetables are cooked is discarded. Vitamin B is not readily destroyed by boiling but is destroyed by heating under pressure.

Vitamin "C."—Vitamin C must be present in the diet, for in its absence animals suffer from scurvy. Scurvy is characterized by soreness of the joints, a spongy condition of the gums, loosening of the teeth and loss in weight. Scurvy has been the bugbear of explorers and navigators for centuries. British ships have been required by act of Parliament to carry a supply of lime juice for the treatment of scurvy and consequently have been nicknamed "lime-juicers." It has long been known that scurvy can be cured by eating raw plants and vegetables. Pine-needle tea and mud baths have been used in the treatment of scurvy. Guinea pigs are very susceptible to scurvy, while rats are immune.

Vitamin C is present in tomatoes, oranges, lemons, apples, potatoes, cabbages and in all fruits and vegetables. Muscle tissue contains but little vitamin C, but more is present in the liver. Vitamin C is slowly destroyed by

boiling, especially in alkaline media. In the presence of acid it is more resistant. Insufficiency of vitamin C is possibly a factor in tooth decay.

Vitamin "D."—A fourth vitamin, D, must be present in the diet of growing animals, for in its absence rickets develops. Rickets is characterized by low values for inorganic calcium and phosphorus in the blood and by a failure of the bones to calcify. When growing animals are kept from exposure to sunlight, or ultra-violet light, they do not develop rickets if the diet contains vitamin D. Animals that are frequently exposed to sunlight do not develop rickets even if the diet is deficient in vitamin D. A diet low in calcium and high in phosphorus, or high in calcium and low in phosphorus, is likely to cause rickets when either vitamin D or sunlight is lacking.

Vitamin D is present in large amount in cod-liver oil, but occurs in butter only in traces. It is present in coconut oil and in green leaves. Steenbock and his collaborators have made the interesting observation that fats that have been exposed to the rays of ultra-violet light for a certain length of time will cure, or prevent rickets. Hess has shown that pure cholesterol acquires antirachitic properties after irradiation. Ordinary cholesterol has no such action.

Vitamin "E."—Vitamin E, which is sometimes spoken of as vitamin X, is necessary for the growth and functioning of the reproductive glands. It is present in plants and is especially abundant in the oil contained in the embryo of wheat.

"Bios."—Still another vitamin, bios, is said to exist and to be necessary for the growth of yeast.

The disease known as pellagra, or rough skin, has been thought by some to be caused by a lack of vitamins, but this has not yet been proved. Pellagra does not occur when the diet contains plenty of fresh meat, eggs and milk. At the present time the etiology of pellagra is unknown.

Dietary Requirement of Vitamins.—The vitamins, although necessary for growth, health, resistance to disease, and for reproduction, are probably required in very small amounts. Polyneuritis in pigeons is cured in a few hours by administering a few milligrams of purified extract of vitamin B.

It is to be expected that when the general public has been made to realize the necessity of including vitamins in the diet and when the supply of these substances becomes available in all seasons and in all regions a considerable number of the ills of humanity will disappear.

Chapter VI

THE ENERGY REQUIREMENT

Calorific Values

The amount of available energy of foods depends largely upon the quantity of fat, carbohydrate and protein present. The calorific values of these substances are as follows:

fat	9.4
carbohydrate	4.1
protein	4.1

These are kilo-calories, or large calories, each of which is equivalent to 1000 small calories. A kilo-calorie is defined as the amount of heat required to raise the temperature of 1 kilogram of water from 15° to 16° C. The values in the table represent the heat produced by the oxidation of 1 gram of material in the animal body. The values for fat and carbohydrate are identical with the values obtained when fats, or carbohydrates, are completely oxidized in a calorimeter, for fats and carbohydrates are completely oxidized by the animal. The proteins, however, are not oxidized completely by the animal, urea being the end product. Urea has a calorific value and this must be subtracted from the calorific value obtained by burning protein in a calorimeter. It is also necessary to make corrections for the heat of solution of urea and for the heat of imbibition of protein.

The Bomb Calorimeter.—Calorific values are obtained by burning substances in a bomb calorimeter. The bomb calorimeter is a heavy steel chamber, lined on the inside with gold, or platinum. It is provided with a plug which can be screwed in to make an air-tight fit. The substance

to be burned is placed within the bomb in a cup, or capsule, in contact with a fine platinum wire connected with the exterior by wiring. Oxygen is pumped into the bomb under pressure. The bomb is placed in a metal cylinder of water. The material in the capsule is then ignited by passing an electric current through the platinum wire within the capsule. The rise in temperature of the bath of water is noted by means of a sensitive Beckmann thermometer. Allowance must be made for the heat absorbed by the metal parts of the apparatus. It is necessary also to make a correction for the heat lost from the apparatus to the outside. This loss of heat can be partly prevented by using insulating material and can be estimated by running check determinations with material of which the calorific value is accurately known. Another procedure is to place the apparatus within a jacket of water and to keep the temperature of this outer layer of water the same as that of the inner layer of water by adding warm water as required. In this manner all loss of heat is avoided. This is known as the adiabatic method.

The Energy Requirement of the Individual.—The energy requirement of the individual naturally depends upon the kind and amount of work which is being done. A clerk working at a desk may require no more than 2800 calories per day, while a farmer doing hard labor in the fields may require as many as 6000 calories. Children require less in proportion to their age, although allowance should be made for the fact that energy is necessary for growth. The average daily requirement of calories for the French nation has been estimated to be 2500 per person, with the requirement for women amounting to 83 per cent of that for men. The study of the calorific values of foods and the relationship of fuel value to cost of production and market price is of the greatest economic importance. The nutritive value of a food depends upon its content of calories, upon the quantity

and quality of proteins present, upon the content of vitamins, minerals and roughage. The palatability and digestibility of a food are of importance also. It can be seen that food which is poorly absorbed is less valuable on that account. One kind of food may be purchased for energy, another for tissue repair and still another for vitamins. Plant foods are cheaper as a source of energy than animal foods and carbohydrates are cheaper than either proteins, or fats. Feeding grain to live-stock is a very wasteful procedure. It has been estimated that if the grain that is fed to raise beef and pork in this country were used directly for human consumption instead, that a very large number of persons could be nourished with the food thus saved.

The Basal Metabolism.—The rate at which the animal produces energy varies in response to stimuli of various sorts. Energy is liberated at a rapid rate during exercise; when the subject is lying prone and motionless the production of energy is at a low level; during sleep energy production is even less. What is known as the basal metabolism is the rate of energy production in an animal lying motionless, though not asleep, at a temperature of 30° C. and in a fasting condition. These conditions have been chosen as standard for the purpose of investigating the effect of age, sex, temperature, disease, drugs, work and nutrition upon the liberation of energy. The metabolic rate can be expressed as calories per hour per kilogram of body weight, but it has been found more satisfactory to express it as calories per hour, per square meter of body surface, for on this basis all animals have been found to have approximately the same basal metabolic rate. This rate is about 40 calories. On the basis of weight the basal metabolism varies inversely with the size of the animal. Small animals produce more heat per kilogram because of their relatively greater exposure of body surface. This is

well illustrated in the table prepared by E. Voit, which is given below:

animal	*cals. per kilo*
horse	11.3
pig	19.1
man	32.1
dog	51.5
goose	66.7
hen	71.0
rabbit	75.1
mouse	212.0

The surface area of the human subject (A) can be obtained from the height in centimeters (H) and the weight in kilograms (W), using the empirical formula of E. F. DuBois and D. DuBois. This is:

$$A = W^{0.475} \times H^{0.725} \times 71.84$$

The Metabolic Rate.—The metabolic rate of warm-blooded animals is increased by cold. In cold-blooded animals the metabolic rate is decreased by cold and the animal eventually becomes torpid. Increase in body temperature, as in fever, causes an increase in the metabolic rate of 10 per cent for every degree Centigrade. This would amount to doubling the rate for an increase of 10° and is in accord with the law that chemical reactions are doubled, or tripled, for every rise in temperature of 10° C.

The metabolic rate is increased by the ingestion of food. In one instance Lusk found that protein caused an increase over the basal rate of 36.7 per cent, fat, 12.7 per cent, and carbohydrate, 5.8 per cent. This is known as the specific dynamic action of foods. Lusk believes that the dynamic effect of proteins is caused by the intermedary products of protein metabolism, such as lactic, pyruvic and glycollic acids. Speakman concludes from his work on bacterial metabolism that the ammonia formed in the deamination of amino acids has a stimulating action on the metabolism and is the cause of the specific dynamic action of pro-

teins. The heating effect of foods should be considered in connection with the season of year. During hot weather protein food should be partaken of sparingly and the diet should contain a large amount of carbohydrate.

The metabolic rate is increased by the injection of adrenalin and thyroxin. The effect of thyroxin is evident in cases of hyperthyroidism, or exophthalmic goiter. In this disease the rate is frequently found to be 50 per cent above normal. On the other hand where there is a deficiency of thyroxin, as in myxedema, or hypothyroidism, the metabolic rate is sometimes as much as 20 per cent below normal.

The metabolic rate is stimulated by exercise more markedly than by any other factor. Severe exertion may cause an increase of as much as 500 per cent. With this effect we are all familiar.

The metabolic rate varies with the age of the subject. DuBois states that the basal rate increases in children from birth until about 5 years of age, when it is 60 calories per square meter of body surface per hour. After 5 years of age the basal metabolic rate decreases rather sharply until the age of 20 years, when it is equivalent to about 40 calories. From then on it decreases very slowly, attaining the value of approximately 35 calories at 85 years of age. The rate in women is lower than in men.

Method of Determination of Metabolic Rate.—The determination of the rate of energy production by animals is of interest and importance both in the study of physiological processes and in the study of disease. It is of especial importance to determine the basal metabolic rate in exophthalmic goiter, because of the danger attending surgical operations on patients in whom the rate is high. Measurement of the basal metabolism must be made at least 18 hours after the ingestion of food and a preliminary rest period of about 40 minutes is necessary to allow the rate to

reach the basal point. During the test complete relaxation is essential. The metabolic rate can be determined by either of two general methods, the direct method and the indirect method.

The Direct Method.—This method depends upon the direct measurement of the amount of heat produced by the subject during a definite interval of time. The subject is placed within an animal calorimeter and the door of the calorimeter is then hermetically sealed. The heat produced by the subject is removed by a stream of cold water circulating through the apparatus. By noting the weight of water passing through the calorimeter box and the difference in temperature between the entering and out-flowing water the heat units can be calculated in calories. To this value must be added the heat lost by evaporation of water from the lungs and skin of the subject. As the animal calorimeter is a rather complicated piece of apparatus the description of the apparatus, together with the numerous corrections involved in the calculation of results, will not be attempted and the student is asked to consult Lusk's "The Science of Nutrition."

The Indirect Method.—The indirect method depends upon the measurement of the amount of oxygen consumed by the subject during a given period of time. It is also necessary to find out what substances have been oxidized during this interval, inasmuch as the calorific value of a liter of oxygen is not the same for fat, carbohydrate and protein. For this purpose the carbon dioxide produced during the experiment is determined. The total nitrogen of the urine is also determined. From the latter value it is possible to calculate how much oxygen has been used up in the catabolism of protein and how much carbon dioxide has been produced by the catabolism of protein. These two values are subtracted from the total oxygen consumed and

the total carbon dioxide produced. The remainders are used to calculate what is known as the non-protein respiratory quotient, which depends upon the relative amount of fat and carbohydrate that has burned. From this data the calorific value of the oxygen consumed in the oxidation of fat and carbohydrate is obtained. To this is added the calorific value for the catabolized protein.

Many types of apparatus are in use for the determination of the metabolic rate by the indirect method. In the closed circuit type the subject is made to breathe a volume of air which is contained in an animal calorimeter. The water vapor is absorbed by passing the air through a sulphuric acid bottle. The carbon dioxide is removed by passing the air through a soda-lime bottle. The air is enriched, as necessary, by the addition of oxygen and then re-enters the calorimeter. A simpler type of apparatus is constructed after the principle of the spirometer. In this apparatus the tank is filled with oxygen. A kymograph drum with a pointer is sometimes used to indicate the volume of oxygen used up.

In the simpler type of apparatus the carbon dioxide is absorbed by soda-lime in order not to interfere with the determination of the oxygen consumed, but the determination of carbon dioxide is usually omitted and the respiratory quotient is arbitrarily taken as 0.78, which naturally makes the results less accurate. However, for clinical purposes the accuracy is sufficient. Determination of the metabolic rate cannot be made with accuracy by estimating the carbon dioxide and neglecting the consumption of oxygen, assuming the respiratory quotient to be 0.78, for carbon dioxide is not always exhaled as rapidly as it is produced, at least over short periods of time, and secondly the calorific value of carbon dioxide varies greatly according to the kind of material oxidized.

In the open circuit type of apparatus the subject is made to breathe outside air. In short experiments the exhaled air may be entirely collected and an aliquot analysed for oxygen and carbon dioxide afterwards. In long experiments aliquots of the expired air are taken from time to time. In any case it is necessary to obtain the volume of the expired air. Analyses of the outside air are also made.

Chapter VII

CONSTITUENTS OF URINE AND THEIR METABOLISM

Physical Properties of Urine

Volume.—The daily volume of urine voided by the normal adult is usually from 800 to 1600 cc., depending upon the amount of liquid ingested and the amount of perspiration. The volume of urine is greater in winter than in summer because hot weather increases the rate of perspiration. In fever and diarrhea and after vomiting the volume of urine excreted is lessened and in acute nephritis the excretion may be very small, or no urine may be excreted at all.

Polyuria, or a condition of increased excretion of urine, may be caused by the ingestion of abnormally large amounts of liquid, but is usually to be regarded with suspicion as it may be caused by diabetes mellitus, or by diabetes insipidus. In both of these diseases the daily excretion of urine is sometimes very great and may amount to more than 10 liters.

Color.—The color of normal urine is a rough index of its concentration. When dilute it is a pale yellow and when concentrated the color is a deep brown. The principal pigment in urine is urochrome, but small amounts of other pigments are also present. These are urobilin, uroerythrin and hematoporphyrin.

In disease the color of urine is sometimes characteristic. Thus, in jaundice the presence of bile pigments may cause the color to be greenish yellow, in poisoning by carbolic acid, in alkaptonuria and in cases with melanotic tumors

the color is brownish, or black. The presence in urine of hemoglobin derivatives, such as hematin and methemoglobin, also gives a brown color, while unaltered hemoglobin colors the urine red.

Certain pigments present in food, or taken as medicines may color the urine. After eating beets the color is sometimes red. The ingestion of santonin causes an orange color; senna and rhubarb produce a reddish brown. After the phenolsulphonephthalein test for kidney function the urine, if made alkaline, becomes colored red.

Transparency.—The urine, when fresh, is clear if acid, but if alkaline is turbid on account of precipitated calcium phosphate. Acid urine may become cloudy from the precipitation of uric acid, urates, mucoid, or nucleoprotein. Urine may be pathologically turbid on account of the presence of fat globules, pus, bacteria, or cells from the urinary tract.

Specific Gravity.—The specific gravity of urine ordinarily varies from 1.015 to 1.025, depending upon the amount of water drunk, the amount of salt in the diet, etc., but in extreme cases may vary from 1.008 to 1.030. In some varieties of kidney disease the kidneys are unable to eliminate salts and consequently the specific gravity is low. In diabetes mellitus the specific gravity may be high, due to a large amount of sugar in the urine. The specific gravity of urine is ordinarily measured by means of a hydrometer, known specifically as a urinometer. Specific gravity is more accurately measured by use of a Westphal balance, or a pyknometer, but great accuracy is not necessary for clinical purposes. As one sound kidney may mask the effect of a diseased kidney, it is sometimes necessary to catheterize the subject and collect the urine separately from each ureter.

Osmotic Pressure.—The osmotic pressure of urine runs parallel with its specific gravity. The pressure is most

easily determined by finding the freezing point of a small quantity of urine, employing for this purpose a sensitive Beckmann thermometer. The sample of urine is immersed in a mixture of salt and ice and is stirred constantly. The moment ice begins to form the temperature of the urine—which has fallen considerably below the freezing point, due to supercooling—rises rapidly to its true freezing point. A reading is made at the instant that the maximum temperature is reached. As the Beckmann thermometer does not give absolute temperatures, it is next necessary to make a reading after placing the thermometer in a vessel containing melting ice. The difference between the two readings is the depression of the freezing point of the urine. This depression is caused by the ions and molecules in solution. Since a depression of the freezing point amounting to 1.85° C. corresponds to an osmotic pressure of 22.4 atmospheres, the osmotic pressure of the sample of urine can be calculated by the equation:

$$\text{osmotic pressure} = \frac{\text{observed depression} \times 22.4}{1.85}$$

The osmotic pressure of urine may vary from two atmospheres to nearly thirty-five atmospheres. This is in contrast with the osmotic pressure of blood, which remains constant at a value, in man, of about seven atmospheres.

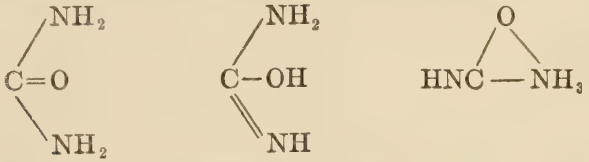
UREA

The most important nitrogenous waste product in man and the other mammals is urea. As we shall see later, in birds and reptiles uric acid replaces urea as the end product of the nitrogen metabolism. In man the urea nitrogen is ordinarily from 85 to 92 per cent of the total nitrogen of the urine. Urea is present in blood, lymph, bile, tears, perspiration, milk and urine; in fact, in all tissues and fluids. This is due to its ability to diffuse readily. In the condition known as uremia, the blood contains a large

amount of urea, sometimes as much as 700 mg. in 100 cc. In a few cases urea has been found in plants.

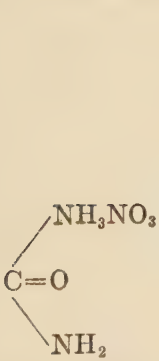
Chemical Character

Urea is a colorless, crystalline solid. It is neutral in reaction and is extremely soluble in water. It is moderately soluble in alcohol. The structural formula usually assigned to urea pictures it as a diamide, but it is probable that urea can exist as the imide and also in a cyclic form:

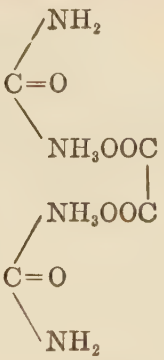


formulas for urea

Urea is rather easily hydrolysed by boiling with dilute acid and alkali, but is more stable than ordinary amides. Urea forms salts with nitric acid and with oxalic acid. These salts are comparatively insoluble in the presence of an excess of acid and give characteristic crystals:



urea nitrate



urea oxalate

Estimation of Urea

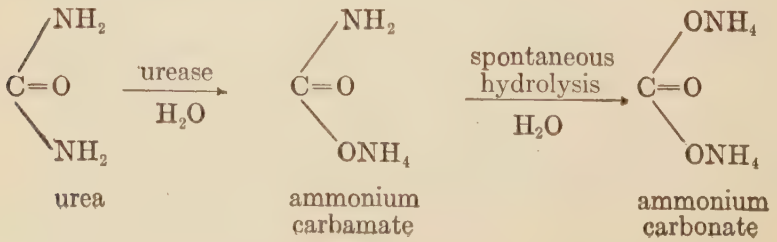
The estimation of urea in blood is of considerable importance for clinical diagnosis, particularly in cases of nephritis. The methods for the quantitative analysis of urea

in blood depend upon the use of the enzyme urease, which is present in the soy bean and in the jack bean. The jack bean is usually employed as a source of urease as it contains approximately sixteen times more urease than the soy bean. In the presence of neutral phosphate solution, urease decomposes urea quantitatively into ammonium carbonate. The ammonia which is formed can be removed by aeration and absorbed by acid. It can then be determined, either by Nesslerization, or by titrating the excess of acid. Urea can be determined in urine, after the removal of ammonia by permutite, by the direct Nesslerization method of Folin and Youngburg. In de-proteinized blood urea can be determined by the direct Nesslerization method of Folin and Sumner (unpublished). The older methods for the estimation of urea gave too high results because the reagents employed reacted not only with urea, but also with other constituents of blood and urine. As an example, in the Knop-Hüfner method the urea is decomposed by sodium hypobromite, giving gaseous nitrogen, which is estimated by volume. The reaction is:

$$CH_4N_2O+3\,NaOBr=3\,NaBr+CO_2+N_2+2H_2O$$

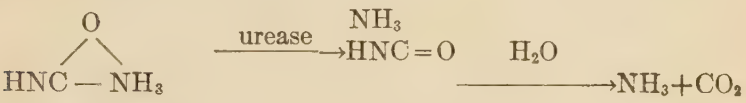
Sodium hypobromite reacts with all compounds containing amino groups in an analogous manner. Hence the results are likely to be too high.

The reaction involved in the conversion of urea to ammonium carbonate by urease is not known. Possibly there is an intermediate formation of ammonium carbamate, the reaction being:



urea — ammonium carbamate — ammonium carbonate

Fearon has advanced the hypothesis that urea is decomposed by urease into ammonia and cyanic acid, the cyanic acid being spontaneously hydrolysed to ammonia and carbon dioxide:



urea (cyclic formula) cyanic acid

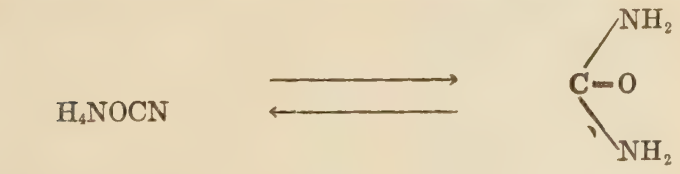
The author objects to this view, inasmuch as the action of urease upon urea takes place in neutral media and cyanic acid, or rather salts of cyanic acid, are not rapidly hydrolysed except in the presence of acid.

As bacteria of many kinds are able to decompose urea into ammonium carbonate, it is necessary to preserve samples of urine and blood, either by keeping on ice, or by the addition of some antiseptic, such as toluene, thymol, or chloroform.

Urea can be estimated by precipitation with xanthydrol. The precipitate is washed, dried and weighed. This method is accurate, but is too time-consuming.

Synthesis of Urea

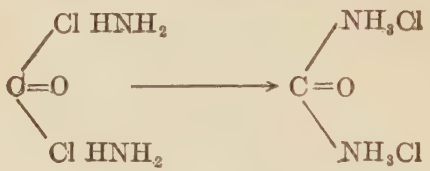
Urea was first synthesized in 1828 by Wöhler. Wöhler was trying to prepare ammonium cyanate by evaporating a solution of potassium cyanate and ammonium sulphate on the steam-bath. He found to his surprise that urea had been formed by an intra-molecular rearrangement:



ammonium cyanate urea

This accidental synthesis of urea was of far-reaching importance because it disproved the idea, which had been held

up to that time, that a mysterious vital force was necessary for the synthesis of all animal and plant compounds. Urea can also be synthesized by passing carbonyl chloride (phosgene gas) into a solution of ammonia:



Formation of Urea

The amount of urea excreted was formerly thought to indicate the condition of the liver, but we now know that it tells only how much protein has been catabolized. There are two sources of urea: the proteins of the food and the proteins of the tissues. If more protein food is eaten, the production and elimination of urea are increased. This is the exogenous or outside source. The endogenous source of urea depends upon the catabolism of the tissue proteins. This process is at a minimum when sufficient carbohydrate is fed, as carbohydrate has a sparing action on the protein breakdown. Under these circumstances the daily production of urea in man eventually sinks to about 5 grams. The endogenous formation of urea is increased whenever the rate of the endogenous metabolism is increased. The endogenous metabolism is increased in fever, after the administration of thyroxin and in arsenic and phosphorus poisoning. When the production of urea is largely endogenous the urea nitrogen amounts to about 60 per cent of the total nitrogen of the urine; when the production of urea is for the most part exogenous the urea nitrogen amounts to more than 90 per cent of the total nitrogen of the urine. From 15 to 30 grams of urea are eliminated daily by the average adult. Human blood normally contains from 9 to 17 mg. of urea nitrogen per 100 cc.

Urea was at one time thought to be formed in the body by the direct oxidation of the proteins. We now believe that the proteins are hydrolysed to amino acids before they are catabolized. Uric acid was at one time thought to be one of the precursors of urea, inasmuch as urea is formed by the oxidation of uric acid by certain reagents. It is now recognized that uric acid is formed by the metabolism of the purines and has no connection with the formation of urea. Creatin likewise has been thought to be a precursor of urea because of the fact that creatin in converted to urea and sarcosin upon heating with alkali. However, creatin does not give rise to urea in the animal body.

The bulk of urea is formed from the amino acids. A very small amount may be formed by the liver from the ammonia of intestinal putrefaction. The amino acid arginin is capable of furnishing a molecule of urea directly on hydrolysis by the enzyme arginase, which is present in the liver. It has been estimated that nearly one-tenth of the urea formed may be produced in this manner. The manner in which urea is formed from the amino acids is not known. At first sight it would seem probable that ammonia might be formed from the amino groups of amino acids by a process of hydrolysis, oxidation, or reduction and that this ammonia would be converted into urea upon reaching the liver, inasmuch as von Schröder has shown that the surviving liver converts ammonium carbonate into urea. However, ammonia is a toxic substance and the amount present in blood is vanishingly small, amounting to 0.1 mg., or even less, per 100 cc. Hence it is by no means certain that ammonia is an intermediate product in the formation of urea. On the contrary, Nash and Benedict believe that the ammonia present in blood is formed from urea by the kidneys. Their hypothesis is founded upon the fact that the blood of the renal artery contains less ammonia than

the blood of the renal vein. They believe that the kidneys produce ammonia from urea for the purpose of neutralizing acids and that some of this ammonia escapes in the venous blood. Bliss has repeated the work of Nash and Benedict and has shown that not only the kidneys, but some other organs as well, have the ability of giving off more ammonia than is brought to them in the arterial blood.

There is some justification for believing that either carbamic acid or cyanic acid may be an intermediate product in the formation of urea. Present evidence on this point is not very convincing.

The formation of urea is of great value to animals. By this process the nitrogen from the catabolized amino acids is converted into a substance that is neutral and harmless. If the waste nitrogen were converted into ammonium carbonate, the body would become alkaline. If the nitrogen were eliminated in the gaseous state, it would be lost to the plant.

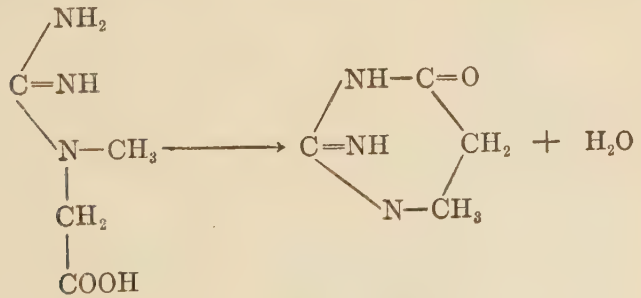
The site of urea formation in mammals has long been believed to be the liver. However, Fiske and Sumner have shown that intravenous injection of amino acids causes an increase in the blood urea that is practically not affected by cutting the liver out of the circulation. It is admitted at the present day that the liver is not the only site of urea formation, nevertheless it is believed by the majority that the liver is the most important organ concerned in this process. One reason for this belief is that the liver has been shown to retain a considerable proportion of the amino acids that are absorbed from the intestine. A more satisfactory reason has recently been brought forward by Myers, Ringer, and Benson, who have shown that when liver tissue is allowed to autolyse a considerable formation of urea occurs, whereas no urea is formed in autolysing muscle tissue.

Creatin and Creatinin

Creatin

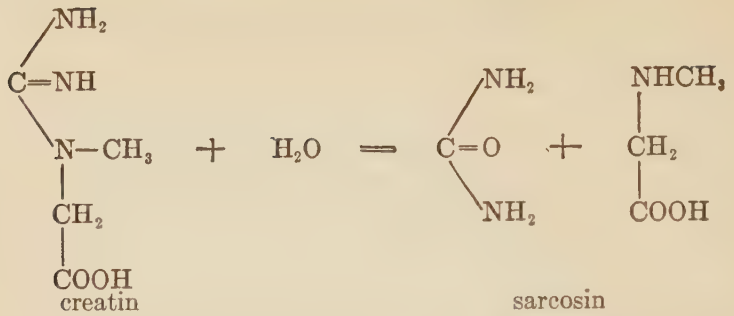
Occurrence.—Creatin, or methyl guanidin acetic acid, is a nitrogenous substance found in vertebrate animals. In voluntary muscles creatin is present to the extent of 0.25 to 0.7 per cent. In involuntary muscles smaller amounts of creatin are present. A small amount of creatin is present in the blood and in the brain. According to Bürger, 98 per cent of the creatin of the body occurs in the muscles. Creatin is called an extractive because it is easily extracted from tissues by water. It is present in the tissues either in a state of adsorption, or loosely combined chemically. Creatin is present in meat soup, gravy, and beef tea and is partly responsible for the bitter taste of these substances.

Chemical Character.—Chemically, creatin can be regarded as derived from guanidin and acetic acid. It is an amino acid, but differs in its metabolism from the other amino acids. It is a colorless, crystalline substance that is readily soluble in water. When boiled with acids it becomes dehydrolysed to form creatinin. This reaction goes practically to completion:



creatin creatinin

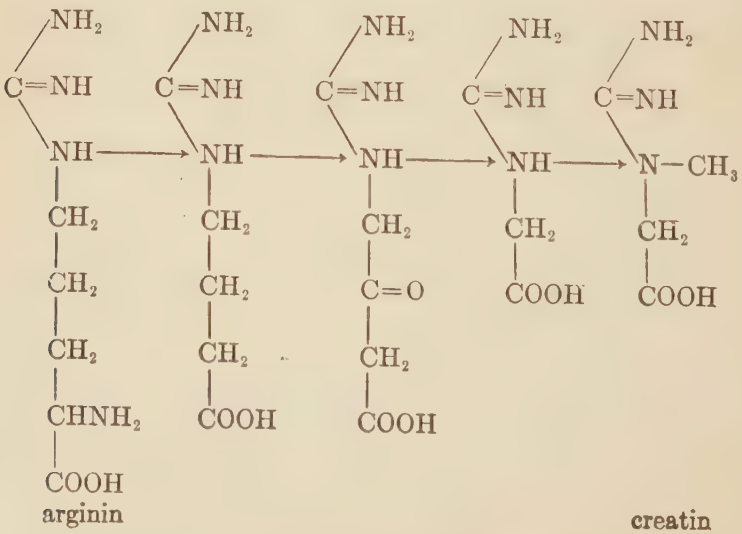
When creatinin is heated with alkali the reverse reaction takes place, but does not continue to completion. Long heating with alkali destroys some of the creatin with the production of urea and sarcosin (methyl glycocoll):



creatin sarcosin

Creatin is not normally present in the urine of adults, but is present in the urine of children before puberty. It appears in the urine during starvation and pregnancy, in fever, exophthalmic goiter, diabetes mellitus, pancreatic diabetes, and phloridzin diabetes.

The presence of creatin in the diet is not necessary as it is readily synthesized by the body. The precursor is presumably one of the amino acids. The presence of the guanidin group in arginin makes this amino acid the most likely precursor of creatin. Creatin might be formed by the following process of oxidation, hydrolysis and methylation:



arginin creatin

Other precursors of creatin that have been suggested are choline and cystin.

Creatinin

Occurrence.—Creatinin, or methyl glycocyamidin, is the anhydride of creatin. Next to urea it is the most important nitrogenous constituent of human urine. About 1 gram of creatinin is eliminated daily by the average adult. On a creatinin-free diet the elimination of creatinin is rather constant. Creatinin is probably present in traces in the blood and tissues, although Behre and Benedict have been unable to isolate creatinin from normal blood. In severe nephritis the blood may contain as much as 30 mg. of creatinin per 100 cc. The values for normal blood are from 1 to 2 mg. per 100 cc.

Chemical Character.—Creatinin is a colorless crystalline substance. It is readily soluble in water and is more basic than creatin. With picric acid and with zinc chloride it forms salts that are moderately insoluble in water. With sodium nitroprusside and alkali creatinin gives a red color. In the presence of sodium picrate and sodium hydroxide, creatinin gives a bright orange color that increases in intensity on standing. This is the *Jaffé test* and has been used by Folin as the basis for the quantitative estimation of both creatinin and creatin. The color is not, as has been sometimes stated, due to the reduction of picric to picramic acid. Greenwald believes the colored compound to be a tautomeric form of creatinin picrate. The author believes the colored compound to be the result of a condensation of creatinin with one, or more, of the nitro groups of the picric acid molecule. This view is substantiated by the fact that creatinin gives a colored compound with sodium nitroprusside and alkali and that creatinin reacts with dinitrosalicylic acid and alkali, as can be demonstrated by the transitory red color produced on acidification.

Acetone decomposes the colored compound which creatinin gives with alkaline picrate and is consequently used by Benedict and Osterberg in their method for the determination of sugar in normal urine.

Estimation of Creatinin.—The determination of creatinin in urine, or in deproteinized blood, is simple. The procedure consists in developing the color by the addition of a solution of pure sodium picrate and alkali. After an interval of about ten minutes the color of the unknown is compared with that obtained from a standard creatinin solution to which proportionate amounts of the reagents have been added at the same time. For the estimation of creatin, the urine, or blood filtrate, is heated with hydrochloric acid in an autoclave. This treatment converts the creatin into creatinin. Both pre-formed creatinin and that formed by the hydrolysis of the creatin are now estimated by means of the color with picrate and alkali. The value for pre-formed creatinin is subtracted from the total, leaving creatin, expressed as creatinin.

The Formation of Creatinin.—The close structural relationship between creatin and creatinin would lead one to believe that the waste product creatinin is continually being formed from the creatin of the muscles. However, Folin found that feeding creatin to animals did not cause an increased excretion of creatinin. Small amounts of creatin when fed were retained and stored in the muscles. When larger amounts of creatin were fed some of the creatin was eliminated unchanged in the urine. Rose and Dimmitt and also Benedict and Osterberg have repeated these experiments, but have used extremely large quantities of creatin. These authors have found that if enough creatin is ingested some creatinin is produced. However the amount of creatinin formed from the ingested creatin is small. It appears probable that creatinin is being continually produced from the creatin present in the muscles, but this process is not materially influenced by the concentration

of creatin present. Creatinin is a waste product and is of no use to the animal. When ingested it is excreted unchanged. Creatin, on the other hand, is a food and when fed in not too great amount is stored for later use. Hammett has demonstated that there exists in muscle tissue a thermostable catalyst which converts creatin into creatinin. This discovery supports the idea that creatin is the precursor of creatinin.

The metabolism of creatin is closely connected with that of the muscles and may be related to their contractile ability. Evidently creatin is necessary for the functioning of the muscle cells. The appearance of creatin in the urine during starvation and in different kinds of diabetes, suggests that the conversion of creatin to creatinin is in some way dependent upon the oxidation of glucose. On the other hand, the appearance of creatin in the urine in fever might be interpreted to mean that creatin is cast off because muscle tissue is being destroyed. The presence of creatin in urine in childhood is difficult to explain. It may be due to the fact that the undeveloped muscle cells cannot utilize creatin as well as adult cells.

The Purines

Nucleoproteins

In the classification of proteins, mention has been made of a class of conjugated, or compound proteins known as nucleoproteins (page 85). These substances are so called because of the fact that they are found principally in the nuclei of cells and are absent from cells, such as human erythrocytes, that possess no nuclei. They are discussed in this place because they are the precursors of the purines, the most important of which is uric acid.

The nucleoproteins can be looked upon as salt-like combinations of a protein with an acid. The protein component is usually of basic nature and is often a protamine,

or a histone. The acid component, or prosthetic group, is nucleic acid.

Discovery.—The nucleoproteins were first discovered by Miescher, who obtained his material from the pus cells of surgical dressings. Hoppe-Seyler later discovered nucleoprotein in the nuclei of yeast cells, and Kossel found nucleoprotein in the nucleated red corpuscles of bird's blood. Nucleoprotein can be obtained from any plant or animal cells that possess nuclei. The spermatozoa of all animals contain nucleoprotein in the heads. Upon adding dilute acetic acid to the spermatozoa, the tails dissolve, leaving practically nothing but nucleoprotein. Plant nucleoprotein can be obtained from ordinary compressed yeast.

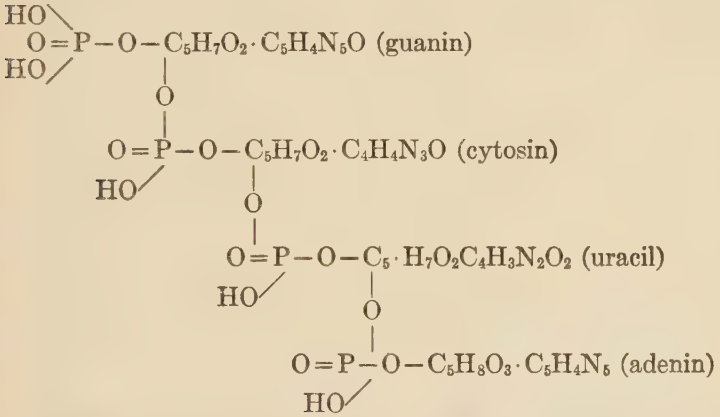
Nature of.—The nucleoproteins are acid in nature and are responsible for the power possessed by the nuclei of cells to stain readily with basic dyes. As the chromosomes of the developing ovum are undoubtedly composed largely of nucleoprotein, the nucleoproteins can be considered to be partly, or wholly responsible for the transmission of hereditary traits and characteristics. Nucleoproteins, according to Jones, have never been isolated in a chemically pure condition and their nature depends upon their manner of preparation. The protein part of the molecule may, or may not, be in part split off, depending upon the method employed. The nucleoproteins always contain from 1 to 1.6 per cent of phosphorus and sometimes they contain iron as well. They are acid in reaction and, unlike the simple proteins, are dextro-rotatory. They are insoluble in water, but dissolve in dilute alkali.

Nucleic Acids.—While many nucleoproteins are found in nature, only two nucleic acids are believed to exist, one in animal nucleoprotein and the other in plant nucleoprotein. As shown below, both animal and plant nucleic acids contain phosphoric acid, adenin, guanin and cytosin, but

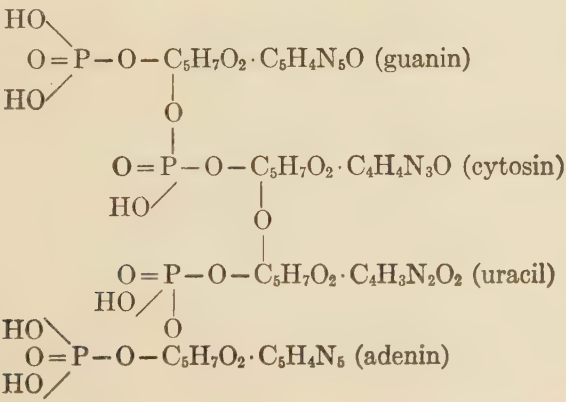
animal nucleic acid contains thymin and a hexose, while plant nucleic acid contains uracil and a pentose.

Animal Nucleic Acid	*Plant Nucleic Acid*
phosphoric acid	phosphoric acid
adenin	adenin
guanin	guanin
cytosin	cytosin
thymin	uracil
hexose	pentose

Levene believes the structural formula for plant nucleic acid to be:



Jones and Perkins have proposed a slightly different formula:



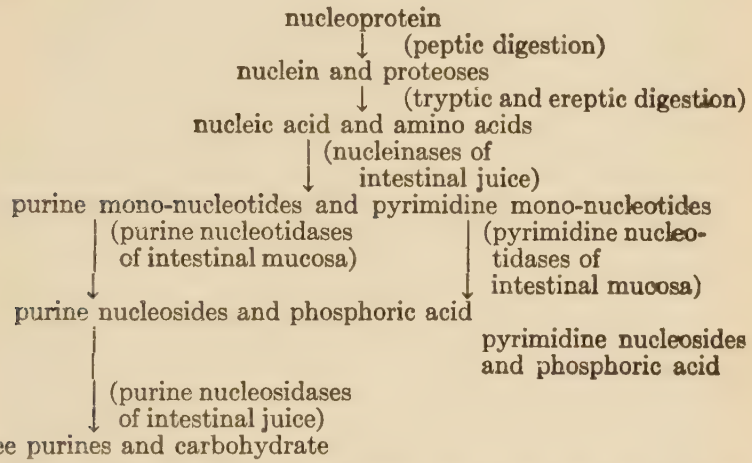
It is evident that considerable doubt still exists as to the exact structural formula of plant nucleic acid. The structure of animal nucleic acid is believed by Levene to be similar to that of plant nucleic acid.

Animal and plant nucleic acids are known as complex nucleic acids, or tetra-nucleotides. Upon hydrolysis by acids, or by enzymes, they are converted into mono-nucleotides, or simple nucleic acids, which contain one molecule each of phosphoric acid, carbohydrate and purine, or pyrimidine. Mono-nucleotides have been isolated from animal tissues and, unlike the complex animal nucleic acid, have been found to contain the pentose sugar d. ribose. One example is inosinic acid, isolated by Liebig from meat extracts. Inosinic acid is composed of phosphoric acid, d. ribose and hypoxanthin. Guanylic acid, isolated by Hammersten from autolysed pancreas, is composed of phosphoric acid, d. ribose and guanin.

Upon ingestion the nucleoproteins are in part decomposed by gastric digestion, with the formation of a substance poorer in protein, known as *nuclein*. The remainder of the protein is split off during pancreatic digestion, with the liberation of the complex nucleic acid.

The intestinal juice contains enzymes known as *nucleinases*, or nucleic acidases, which decompose the complex plant and animal nucleic acids to simple nucleic acids, or mono-nucleotides. Intestinal juice also contains an enzyme known as *nucleotidase*, which acts upon mono-nucleotides containing purines (see p. 171) with the liberation of phosphoric acid and *nucleosides*, which are compounds containing purine united with carbohydrate. Enzymes attacking the pyrimidine nucleotides are not present in the intestinal juice, but are found in the intestinal mucosa. The intestinal mucosa contain also enzymes known as *nucleosidases*, which hydrolyse the purine nucleosides, with the liberation of free purine and carbohydrate.

Enzymes attacking the pyrimidine nucleosides have been found neither in the intestinal mucosa, nor in animal tissues as a whole. The course of the enzymic decomposition of ingested nucleoprotein is illustrated by the following diagram:


nucleoprotein
(peptic digestion)
nuclein and proteoses
(tryptic and ereptic digestion)
nucleic acid and amino acids
(nucleinases of intestinal juice)
purine mono-nucleotides and pyrimidine mono-nucleotides
(purine nucleotidases of intestinal mucosa)
(pyrimidine nucleotidases of intestinal mucosa)
purine nucleosides and phosphoric acid
pyrimidine nucleosides and phosphoric acid
(purine nucleosidases of intestinal juice)
free purines and carbohydrate

The nucleoproteins of the body cells are believed to be hydrolised in the process of endogenous metabolism by the same, or by similar enzymes, with the ultimate formation of the same products. Although the action of pepsin and trypsin is replaced in the tissues by that of tissue erepsins, we find that nucleinases, purine nucleotidases, pyrimidine nucleotidases and purine nucleosidases are generally distributed in animal tissues.

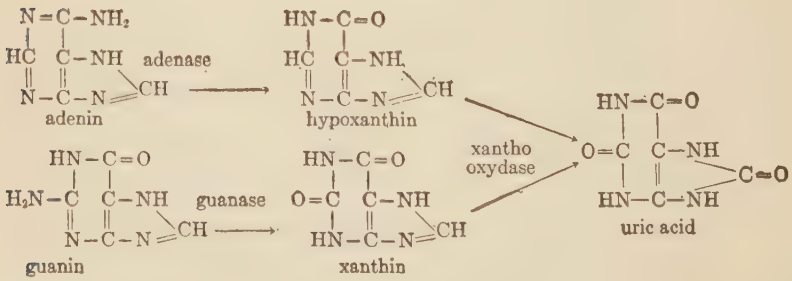
The nucleoproteins have been definitely shown not to be necessary for the purposes of nutrition, as they are readily synthesized by animals from amino acids. Eggs contain practically no nucleoprotein, or purine, when first laid, but during the process of incubation their content of nucleoprotein greatly increases. The salmon eats no food during the spawning season, but nevertheless synthesises a large amount of nucleoprotein for the production of spermatozoa,

using its own tissue proteins for this purpose. Young mammals fed with milk, which is largely free from nuclear material, rapidly synthesize nucleoprotein. It has been shown that rats can grow well on diets containing no purines, or nucleoproteins.

Ackroyd and Hopkins have shown that either arginin or histidin can be used by the animal for the synthesis of nucleic acids.

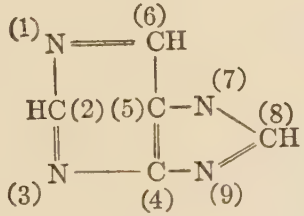
Uric Acid

Horbaczewski's Experiment.—A classical experiment dealing with the metabolism of the purines was performed by Horbaczewski, who bubbled oxygen through putrefying spleen pulp and found uric acid to be produced. It has been shown that in this process the original material consists of the purine bases adenin and guanin and that putrefaction is not necessary, as Horbaczewski at first believed. The spleen, as well as many of the other organs, contains the enzymes adenase and guanase which are able, in the presence of a sufficient supply of oxygen, to deaminize and oxidize adenine to hypoxanthin and guanin to xanthin. The intermediate products, hypoxanthin and xanthin, are in turn attacked by a third oxydase, known as xantho-oxydase, and are transformed into uric acid. The reactions are



The purine oxydases have been found to be rather irregularly distributed in such organs as the liver, spleen, pancreas and kidneys. Inasmuch as adenase is said not to be present in human tissues and guanase is either lacking or is present only in small amount in pigs, it seems possible that some uric acid may be formed in the animal in a manner other than the one described above.

Purine.—Uric acid is the end product of the purine metabolism in man. Chemically uric acid and all of the purines can be considered to be built from the skeleton known as the purine ring. The compound known as purine, which was first synthesized by Emil Fischer, is shown below:



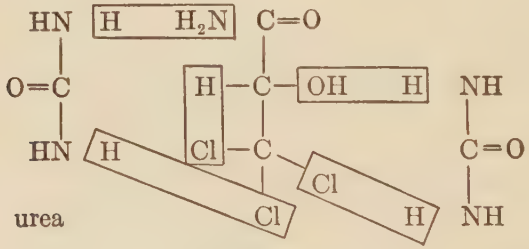
purine

It will be noted that *the purine ring* is formed by a fusion of the six-membered pyrimidine ring with the five-membered imidazol ring. The atoms of the ring have been numbered by the organic chemist in order to simplify the terminology of the purines and the writing of their structural formulas. Thus, adenin is 6 amino purine, guanin is 2 amino 6 oxy purine, while uric acid is 2,6,8, trioxy purine. In writing the structural formula of one of the purines the student is advised to write first the purine ring and to add such groups as are expressed in the technical name. Next, double bonds should be added, if possible, in those positions where double bonds occur in purine itself. Finally, hydrogen atoms should be added wherever it is possible.

Synthesis of Uric Acid.—The synthesis of uric acid has been achieved in a variety of ways. When pseudo uric acid is boiled with dilute mineral acids, uric acid is formed by the elimination of a molecule of water:

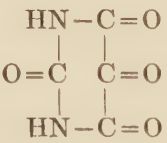
pseudo uric acid — uric acid

Uric acid is formed when urea is fused with glycocoll and also when urea is heated with trichlor lactamide:



trichlor lactamide

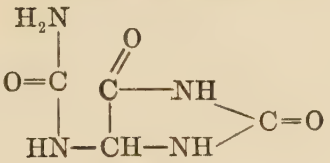
When uric acid is heated with hydrochloric acid in a sealed tube, it is decomposed to form glycocoll and ammonium carbonate. Oxidation in the cold with nitric acid gives alloxan:



aloxan

Oxidation with potassium permanganate converts uric acid to *allantoin,* which is of considerable importance, because it is produced by the oxidation of uric acid in most

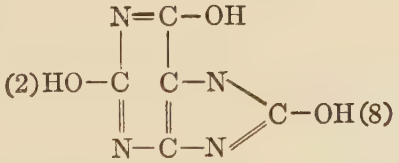
of the mammals. Allantoin is present in the allantoic fluid, in the urine of the mammalia and in certain plants. It occurs in human urine only in traces.



allantoin

Uric acid is decomposed by bacteria and also by hydrogen peroxide, with the formation of urea.

Properties of Uric Acid.—Uric acid is to be distinguished from the purine bases on account of its acid properties. It is an odorless, micro-crystalline substance. Its most distinguishing characteristic is its great insolubility in water; 1 part of uric acid is soluble in 40,000 parts of water at 18° C. Uric acid is soluble in alkali, in concentrated sulphuric acid and in piperazine (diethylene diamine). It is a very weak acid. On account of its acid properties it is believed to be capable of keto-enol isomerism. The usual structural formula for uric acid is known as the lactam form, *the lactim form* is given below:



With this structure the hydrogen atoms in positions 2 and 8 are replaceable by cations. Hence uric acid will form two series of salts. The monobasic salts are relatively insoluble, while the dibasic salts are much more soluble. Of the latter the lithium salt is the most soluble and lithium carbonate is usually chosen when it is necessary to dissolve uric acid for the preparation of standard solutions. Uric acid in

alkaline solution is easily oxidized by atmospheric oxygen and it is therefore best to avoid an excess of alkali.

Determination of Uric Acid.—Uric acid can be *qualitatively tested* for by means of the murexide test, which consists in heating gently some of the solid substance with strong nitric acid. After cooling, the addition of ammonia produces a red, or purplish color. A much better test depends upon the ability of uric acid to reduce phosphotungstic acid in alkaline solution to an intensely blue colored compound. This reaction is the basis of the Folin method for the *quantitative determination* of uric acid in blood and urine. In this method the uric acid is first separated from interfering substances by precipitation as the silver salt. After centrifuging, the silver urate is decomposed by adding sodium cyanide. In recently described methods for the determination of uric acid in blood and urine (Benedict), and in blood (Folin), the preliminary isolation is dispensed with. Benedict uses arseno-phosphotungstic acid in place of phosphotungstic acid. Morris uses arsenotungstic acid.

The determination of uric acid in blood is of importance in the diagnosis of gout, as this disease is characterized by increased values. Normal blood contains 2–3 mg. per 100 cc. The blood of most mammals contains the merest traces of uric acid because nearly all of the mammals possess the power of oxidizing uric acid to allantoin.

Elimination of Uric Acid.—About 0.6 gm. of uric acid is eliminated in the urine daily by the average adult. On a purine-free diet of milk, eggs, bread and vegetables the excretion of uric acid may sink to 0.3 gm. per 24 hours. Glandular organs, such as liver, pancreas, thymus, and kidneys, are rich in nuclear material and the addition of these to the diet considerably increases the production and elimination of uric acid. Not all of the ingested material reappears as uric acid, however, as some of the purine may

be destroyed in the intestinal tract by bacteria and some of the uric acid produced is destroyed in the circulating blood, according to Folin. Non-purine foods have been found to cause a slight increase in the elimination of uric acid within 2–3 hours after their ingestion. The reason for this is obscure. It has been suggested that it is due to the increased metabolism of the cells of the digestive system, with a consequent increase in the rate of the endogenous purine metabolism of those cells.

Source of Uric Acid.—It can be readily seen that like many other end products of the metabolism, uric acid has two sources. A rather constant amount is being produced continually from the nucleoproteins of the tissues. This is the endogenous source. The exogenous uric acid is formed from the nucleoprotein and purine material present in the food and varies in amount, depending upon the kind and amount of food eaten. In children the uric acid production is relatively high because, as Folin has suggested, their metabolism is more intense than that of adults. In leukemia, in recovery from pneumonia, after prolonged exposure to X-rays and after severe burns, there occurs an increased production of uric acid. This is on account of the destruction of cells containing nuclear material.

Birds and Reptiles.—In birds and reptiles uric acid is produced in large amount, as it is formed not only, as in man, from the catabolism of the purines, but also from the proteins. According to A. P. Mathews, the reptiles originated in a desert and, together with their descendants the birds, are provided with the power of excreting their nitrogenous waste in solid form in order to avoid losing water. Uric acid is capable of excretion in solid form on account of its insolubility.

Minkowski performed a *celebrated experiment* by extirpating the livers of geese and showing that during the short period of time that the geese survived this operation

they excreted chiefly ammonium lactate instead of uric acid. The small amount of uric acid excreted after the operation was thought to come from the purine metabolism. Minkowski's experiment proved that the liver of the bird is the site of a synthesis of uric acid. It has been found that perfusion of the surviving goose liver with ammonium lactate leads to a synthesis of uric acid. When ammonium salts of 3 carbon atom acids, or urea, are fed to normal geese, the production of uric acid is found to be increased.

Primates vs. Mammals.—In man and the anthropoid apes, and also in the Dalmatian coach dog, uric acid is very largely the end product of the purine metabolism, while in other mammals it is merely one of the intermediate products. The similarity between the purine metabolism of man and the anthropoids (gibbon, orang-outang, chimpanzee, gorilla, etc.) is good evidence of the correctness of the theory of evolution. The case of the Dalmatian coach dog is anomalous. The tissues of ordinary dogs and of all other mammals, with exception of the primates, contain the enzyme uricase, otherwise known as the uricolytic enzyme. In the presence of oxygen uricase oxidizes uric acid to allantoin and this compound is found in the urine. Traces of allantoin are present in human urine and this fact tends to support the recent claims of Folin and Berglund that a variable amount of uric acid is oxidized in the human subject.

Gout.—It has been suggested that the lack of uricase in human tissue is an explanation of the occurrence of gout. Inasmuch as pigs suffer from guanin gout, a parallelism has been drawn between the absence of uricase in man and the absence of guanase in the pig. The lack of uricase, however, is only one of the many factors to be considered. Folin, Berglund and Derick believe that in gout there is a faulty elimination of uric acid by the kidney and that this faulty elimination is supplemented at times by a decreased

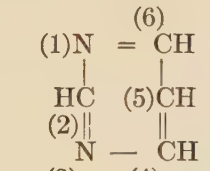
destruction of uric acid. Myers and Fine have pointed out that uric acid is one of the substances least readily eliminated by the diseased kidney.

Other Purines in Urine

Human urine contains other purines besides uric acid, but in traces only. Some adenin, xanthin and hypoxanthin that have escaped destruction in the tissues are present. Other purines present are paraxanthin, episarkin, heteroxanthin, epiguanin and I methyl guanin. The presence of these purines is the result of the ingestion of the vegetable purines caffeine, theobromine and theophylline. Caffeine, or 1,3,7 trimethyl, 2,6 dioxy purine, is present in coffee, tea, maté and coco cola. As it is a poison, the animal detoxicates it by splitting off some of the methyl groups. Theobromine, present in cocoa, is 3,7 dimethyl, 2,6 dioxy purine; while theophylline, or 1,3 dimethyl, 2,6 dioxy purine, is found in tea. The detoxication of these substances is not complete and they appear in the urine in part unchanged.

Pyrimidines

The pyrimidines contain the pyrimidine ring, which is present as a sub-structure in the purine ring. The compound pyrimidine is shown below with the atoms of the ring numbered in the usual manner:



pyrimidine

Only three pyrimidines are found in nucleoproteins. *Cytosin,* or 2 oxy, 6 amino pyrimidine, is a constituent of both plant and animal nucleic acids. *Uracil,* which occurs

only in plant nucleic acid, is 2,6 dioxy pyrimidine. *Thymin* owes its name to the fact that it was first isolated from the thymus gland. It occurs only in animal nucleic acid. It is 5 methyl uracil.

Practically nothing is known about the metabolism of the pyrimidines. As pyrimidines are not found in the urine, they are believed to be oxidized in the body. However, when free pyrimidines are fed to animals these compounds are not destroyed, but are eliminated unchanged. This evidence, coupled with the fact that animal tissue contains no enzyme (pyrimidine nucleosidase) capable of hydrolysing the pyrimidine nucleosides, leads one to believe that to be oxidized the pyrimidines must exist as nucleosides and not as free pyrimidines.

The Acidity of Urine

The Effect of Diet

The urine of carnivora is habitually acid in reaction, while that of herbivora is alkaline. This difference is caused by a difference in diet. During starvation the urine of herbivorous animals becomes acid as the starving animal is subsisting upon its own flesh and has become carnivorous, in an endogenous sense. The catabolism of food, or tissue proteins and phosphatides, gives rise to sulphuric and phosphoric acids. Sulphuric acid is produced by the oxidation of the two sulphur-containing amino acids. Phosphoric acid is present in nucleoproteins, phosphoproteins and phosphatides as an ester and is liberated as the free acid in the catabolism of these compounds. The two acids are neutralized as soon as produced by the alkali of the tissues and blood and their effect is to decrease the supply of alkali in the body.

The diet of herbivora consists of grasses and other vegetable materials which contain small quantities of proteins and large amounts of organic acids and acid potassium salts

of these organic acids. During catabolism the organic acids are completely oxidized to carbon dioxide and water, leaving a residue of potassium bicarbonate. As carbon dioxide is readily eliminated by the lungs, the effect of the potassium bicarbonate is to make the blood and tissues more alkaline. The excess of alkali is secreted by the kidneys.

Acids of the Urine

In man the reaction of the urine is nearly always acid. According to Henderson and Palmer the pH of human urine may vary from 4.8 to 7.4. The average is about 6.0. Whatever may be the acids produced in the cells, the acid substances present in urine are acid phosphates together with organic acids. Some of these organic acids are: carbonic, uric, hippuric, benzoic, lactic, and oxalic. In starvation aceto-acetic and beta oxybutyric acids are present in the urine in considerable amounts. In severe diabetes the quantity is very large.

The Alkaline Tide.—The reaction of urine varies considerably from hour to hour. After meals the reaction is always shifted toward the alkaline side. This phenomenon, which is known as the alkaline tide, is caused by the gastric secretion of hydrochloric acid, which results in a liberation of alkali into the blood. The reaction that occurs in the gastric mucosa is possibly:

$$NaCl + NaH_2PO_4 = HCl + Na_2HPO_4$$

The hydrochloric acid is excreted into the lumen of the stomach, while the di-basic phosphate is absorbed into the blood. The increased alkalinity of the blood is reflected in the urine.

In cases of intestinal obstruction, where there is vomiting, considerable quantities of hydrochloric acid are lost in the vomitus. The resulting increase in the alkalinity of

the blood may become so great as to cause death by alkalosis.

The occurrence of the alkaline tide after meals might well be used as a clinical demonstration of the secretion of hydrochloric acid. According to Hubbard, no alkaline tide occurs in cases of gastric achylia.

It is possible to increase the alkalinity of the urine by increasing the rate of removal of carbon dioxide by the lungs. This may take place as the result of forced breathing, hot baths, or the subjection of an animal to a rarefied atmosphere. The excessive loss of carbonic acid leads to an increase of the alkalinity of the blood and the kidneys immediately begin to secrete a more alkaline urine.

Increased Acidity.—After severe exercise the urine often becomes more acid on account of the production of lactic acid by the tissues. Lactic acid is ordinarily oxidized as rapidly as it is formed, but when muscular effort is severe the supply of oxygen is not always sufficient.

Amount of Acid Secreted

The amount of tenth normal acid that can be secreted by the human kidneys in a 24-hour period amounts to more than 1000 cc. On an average diet the amount is from 200 to 500 cc. This production of acid is small in contrast with the amount of carbonic acid that is eliminated by the lungs. According to D. W. Wilson the lungs remove from 20 to 40 liters of normal acid daily.

The kidneys are unable to secrete strong acids, but by producing ammonia from urea are able to conserve the body's supply of base. The ammonia present in the urine is neutralization ammonia, and the amount present is indicative of the amount of acid that has been excreted. On a high protein diet the urinary ammonia is increased in consequence of the increased production of sulphuric and phosphoric acids. In diabetic acidosis the ammonium

nitrogen may amount to 40 per cent of the total urinary nitrogen. If a mineral acid, such as hydrochloric, is fed to animals, there occurs an increased output of ammonia. On the contrary, when sodium bicarbonate is given the neutralization ammonia vanishes. When ammonium chloride is ingested, it reappears as such in the urine; if an ammonium salt of an organic acid is fed no ammonia reappears in the urine, for the acid radical is completely oxidized and the ammonia is converted into urea.

Titration Acidity.—The production of acid by the kidneys can be quantitatively determined by titrating urine with standard sodium hydroxide, using phenolphthalein as indicator. As phenolphthalein changes color on the alkaline side of neutrality a titration value is given even with urine that is alkaline. Calcium salts interfere with the titration and are therefore eliminated by the addition of potassium oxalate. The interfering action of calcium salts is due to their reaction with di-basic phosphates, whereby tri-calcium phosphate precipitates with the simultaneous production of acid. The reaction is:

$$3CaCl_2 + 2Na_2HPO_4 = Ca_3(PO_4)_2 + 4NaCl + 2HCl$$

Ammonium salts interfere somewhat with the end-point.

H Ion Concentration.—Determination of the hydrogen ion concentration of urine has some clinical value as an indication of the presence of acidosis. In cardio-renal disease the pH is about 5.3, in diabetic acidosis the acidity is greater than this. In certain cases of nephritis the urine has been found to be no more acid than usual in spite of the fact that an alarming state of acidosis may exist. Here the kidneys are evidently unable to secrete acids, or are unable to produce neutralization ammonia.

The determination of the hydrogen ion concentration should be carried out on urine immediately after it is voided as the free carbonic acid present in the urine is lost rather

rapidly and the urine consequently becomes more alkaline on standing. The indicator method is usually used in this determination. The indicators employed are alizarin red, phenol red, methyl red, nitro-amino guaiacol, p. nitro phenol, brom-cresol purple and brom-thymol blue, depending upon the range of reaction to be covered. The indicator is added to the urine and the color is compared with that of buffer standards of known pH, containing the same concentration of indicator as the urine. The yellow color of the urine interferes with the comparison, but this factor can be eliminated by placing a tube containing urine with no indicator between the source of light and the standard tube.

Fermentation.—Urine that contains no preservative soon undergoes an alkaline fermentation, owing to the action of bacteria upon the urea and the resultant formation of ammonium carbonate. In cystitis the urine may ferment in the bladder. The acid fermentation of urine is possible, though rare.

Sulphur

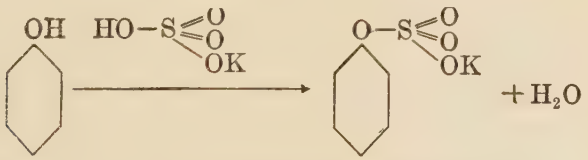
Sources

Sulphur is ingested chiefly in organic combination as the sulphur of proteins. Much of this sulphur is contained in the amino acid cystin and it is possible that some may be present as cystein sulphur, but a part exists in proteins in an entirely different sort of linkage, as it is not split off on heating with alkali like the sulphur of cystin. The new amino acid isolated by Mueller from casein, egg albumin, edestin, wool and gelatin is apparently responsible for this firmly bound sulphur. In the glycoproteins (mucins, mucoids and chondroproteins), we find sulphur present in such combinations as mucoitin and chondroitin sulphuric acids.

Sulphur Metabolism

During digestion the putrefactive bacteria convert some of the sulphur into hydrogen sulphide and mercaptans. In the process of metabolism the sulphur of the two sulphur-containing amino acids is oxidized to sulphuric acid, which is neutralized by the bases of the body to form ammonium, sodium, potassium, calcium, or magnesium sulphates. These inorganic sulphates are waste products and are eliminated in the urine. From 75 to 80 per cent of the total sulphur excreted is in this form. The reactions involved in the oxidation of sulphur are not known. Lewis and Root have shown that when the amino groups of cystin are blocked, as in phenyluramino cystin, that oxidation of the sulphur is prevented.

A second class of sulphur compounds present in the urine is known as ethereal sulphates. These are largely detoxication compounds and are the result of the production in the intestine of such compounds as phenol, para cresol, indol and skatol by bacterial putrefaction. Upon absorption these bacterial products are detoxicated by the cells and are converted into ethereal sulphates, in some cases with preliminary oxidation. As an example the formation of phenyl potassium sulphate is given below:



phenol phenyl potassium sulphate

A third class of sulphur compounds, present in the urine, is known as unoxidized, or neutral sulphur. This class of sulphur compounds is comprised of such substances as cystin, thiocyanates, sulphonic acids, urochrome, oxyproteic acid, uroferric acid, etc.

Quantitative Determination

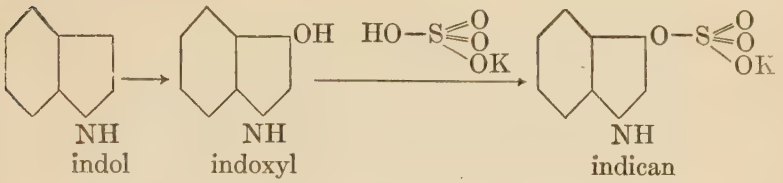
The quantitative estimation of inorganic sulphates in urine is dependent upon their precipitation either as barium sulphate, or as benzidine sulphate. The precipitated barium sulphate is washed, dried, ignited and weighed. The benzidine sulphate is washed and titrated with sodium hydroxide.

Ethereal sulphates are not precipitated by barium, or by benzidine, and are usually determined together with the inorganic sulphates after hydrolysis with hydrochloric acid. Acid hydrolysis converts ethereal sulphates into the organic radical and free sulphuric acid. The difference between inorganic sulphates and total sulphates represents the ethereal sulphates.

The neutral sulphur of urine can be determined by subtracting the value for total sulphates from the value for total sulphur. Total sulphur is determined by oxidizing an aliquot of urine with some oxidizing reagent. The sulphuric acid is then precipitated by barium, as usual. Since the amount of ethereal sulphur and of neutral sulphur is small, it has been suggested that more accurate results can be obtained by determining these classes of sulphur compounds directly. This can be done by first removing the inorganic sulphates, in the case of the determination of ethereal sulphates, and by first removing the total sulphates, in the case of the neutral sulphur compounds.

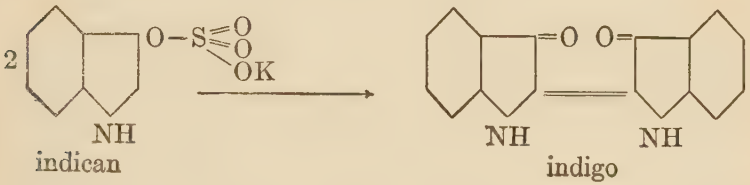
Indican.—One of the ethereal sulphates present in urine that is sometimes determined quantitatively is *indican,* or potassium indoxyl sulphate. Indican is the detoxication product of indol, which is formed by the action of bacteria upon tryptophan in the intestinal tract. The indol which is absorbed is converted by the cells first into the oxidation

product, indoxyl, which is then esterified with sulphuric acid to form indican:



In this manner a lipoid, or fat-soluble substance, is changed into a water-soluble substance and the poisonous action is removed.

To detect indican a sample of urine is treated with strong hydrochloric acid and some oxidizing agent, such as ferric chloride, causing the formation of indigo:



The indigo is shaken out with chloroform and determined either colorimetrically, or by titration with permanganate.

Taurin.—The taurocholic acid of the bile is composed of cholic acid and the substance *taurin:*

$$\begin{array}{l} CH_2SO_3H \\ | \\ CH_2NH_2 \end{array}$$

taurin

Taurin is presumably formed in the body by the oxidation of cystein, but cannot be considered to be an intermediate product in the main catabolism of cystin as taurin does not give rise to inorganic sulphates when it is fed to animals (Lewis).

Effect of Diet on Sulphur Excretion

The excretion of sulphur in the urine is a criterion of the protein metabolism and the total sulphur, expressed as SO_3, is about one-fifth as great as the total nitrogen. However, the elimination of sulphur is not so constant as the elimination of nitrogen, because the sulphur content of proteins varies considerably more than the content of nitrogen. When the subject changes from a high to a low protein diet the inorganic sulphates in the urine diminish relatively and absolutely. This shows that the inorganic sulphates are partly endogenous and partly exogenous. The catabolism of food protein gives rise to exogenous sulphates and the catabolism of tissue proteins gives rise to endogenous sulphates.

Change from a high to a low protein diet causes the ethereal sulphates to decrease absolutely, but to increase relatively. The absolute decrease in the production of ethereal sulphates shows that intestinal putrefaction is decreased by a diet low in protein. The relative increase indicates that ethereal sulphates either arise partly from the endogenous metabolism, or else that intestinal putrefaction still occurs on a low protein diet.

When the subject changes from a high to a low protein diet the neutral sulphur is not affected absolutely, but increases relatively. This shows that neutral sulphur is purely a product of the endogenous metabolism.

Cystin.—In the metabolic derangement known as cystinuria, *cystin* is eliminated in the urine in considerable amount. This cystin arises from the endogenous metabolism. Cystin that is fed to cystinurics is usually well oxidized and does not reappear in the urine. The cause of cystinuria is unknown.

Cystin has been found by Osborne and Mendel to be essential for growth. It is also of importance as a catalyst.

The substance glutathion is composed of one molecule of cystin and two molecules of glutamic acid. Upon reduction the cystin group is split with the formation of two cystein groups and the molecule is divided into two parts. Upon oxidation the two halves are again re-united through the sulphur atoms. In this manner glutathion acts as a carrier of hydrogen, or as a potential carrier of oxygen.

Sulphur is thought to be used by the animal for the detoxication of cyanides. The product of detoxication is the thiocyanate ion. Potassium thiocyanate is present in saliva and in urine in small amount. In dogs *cystein* is used for the detoxication of brom benzene.

Phosphorus

Sources

Phosphorus is present in food materials largely in organic combination in such compounds as phosphoproteins, nucleoproteins and phosphatides. The phosphorus is present as orthophosphoric acid and exists in ester form. Small quantities of inorganic phosphates are present in meat, grains and vegetables. Milk contains a considerable amount of inorganic phosphorus as calcium phosphate.

Metabolism

The absorption of phosphorus is not complete and a considerable portion of the ingested phosphorus is present in the feces as tri-calcium phosphate. The phosphoric acid which is liberated in the cells in the process of metabolism is at once neutralized by bases and is eliminated in the urine as inorganic phosphate. Inorganic phosphates constitute nearly all of the phosphorus in the urine. A small amount of organic phosphorus is present in urine as glycerophosphoric acid, phosphocarnic acid, and the like. The

inorganic phosphates of acid urine are largely primary phosphates of the type MH_2PO_4. Some secondary phosphates may be present in faintly acid urine. When urine is neutralized, or made alkaline, tri-calcium phosphate, $Ca_3(PO_4)_2$, precipitates. During the alkaline tide the urine is likely to contain a precipitate of calcium phosphate. This phenomenon is called phosphaturia, but it is without significance except as regards the reaction of the urine. About 60 per cent of the inorganic phosphates in urine are salts of sodium, potassium and ammonium. The remainder is present as salts of the alkaline earths. Upon boiling urine that is only slightly acid in reaction, a precipitate of tri-calcic phosphate often appears. This precipitate interferes with the heat coagulation test for albumin, unless dissolved by the addition of dilute acetic acid. Tri-calcic phosphate is probably sometimes held in solution in the urine by the presence of carbonic acid or else it is present as a supersaturated solution.

The amount of phosphorus in the diet is usually sufficient to meet the requirements of the animal. Inorganic phosphate has been found to be as well utilized as organically combined phosphorus. The absorption of phosphates is somewhat dependent upon the amount of calcium and magnesium in the food. The concentration of phosphorus in the blood in adults is about 3.7 mg. per 100 cc. In children the amount is about 5.6 mg. After bone fracture the blood phosphorus is temporarily increased. In cases of rickets the blood phosphorus is likely to be low. In such cases the administration of cod liver oil and irradiation by ultra-violet light raises the value of the blood phosphorus.

The phosphorus compounds of the blood can be divided into three classes: protein, lipoid and acid-soluble. The acid-soluble fraction includes inorganic phosphates and organically combined phosphates. The latter are present largely, if not entirely, in the erythrocytes and have been

shown by Greenwald to consist for the most part of diphospho-l-glyceric acid.

Elimination

The elimination of phosphorus in the urine depends upon the amount of phosphorus in the diet, but is a rather variable factor. After the ingestion of carbohydrate the excretion of phosphate is decreased, as Fiske has shown. This phenomenon indicates that phosphate is used in the catabolism of glucose and is in accord with the theory that glucose di-phosphoric ester is an intermediate in the oxidation of glucose in animals as well as in yeast. The average daily excretion of phosphorus by man is about 2.5 grams, expressed as P_2O_5. After parathyroidectomy the excretion of phosphorus is decreased; and the blood phosphorus is decreased also.

Quantitative Determination

There exist many methods for the quantitative determination of phosphorus in blood, urine, and other biological materials. The choice of the method to be employed depends upon the accuracy required, the quantity of material available for analysis, the number of analyses to be run, etc. The principles of several of the quantitative methods for the determination of phosphorus are outlined below:

1. The phosphate is precipitated as ammonium phosphomolybdate in acid solution. This precipitate is washed and is either: treated with an excess of alkali and the excess titrated back with tenth normal acid; or the precipitate is dissolved in ammonia and the phosphorus is precipitated by adding magnesia mixture as magnesium ammonium phosphate. The precipitate is washed, ignited and weighed as magnesium pyrophosphate.

2. The phosphate solution is titrated hot with uranium

acetate solution. When an excess of uranium is present this fact is indicated by a brown color given when a drop of the mixture is treated with a drop of a ferrocyanide solution.

3. The phosphate solution is treated with a solution of molybdic acid in sulphuric acid. Phosphomolybdic acid is formed. Upon adding 1, 2, 4 amino naphthol sulphonic acid the phosphomolybdic acid is reduced to the intensely blue reduction product. The blue color is read in a colorimeter against a standard.

4. The phosphate solution is treated with molybdic acid. Then a solution of strychnine is added, giving a suspension of strychnine phosphomolybdate. The suspension is read in a nephelometer against a standard.

Chlorides

Chlorides in the urine are in inorganic form and may be considered to be derived very largely from the sodium chloride of the diet. Expressed as sodium chloride the excretion in man amounts to from 10 to 15 grams per day, making sodium chloride—next to urea—the most important constituent of the urine. The large elimination of sodium chloride in human urine indicates that an unnecessarily large amount of salt is eaten. One or two grams of salt per day would be amply sufficient for all purposes of nutrition. An excess of salt in the diet simply means more work for the kidneys. In nephritis the ingestion of salt is sometimes harmful. Uncivilized tribes that live largely on meat seem not to desire salt in their diet. Stefansson states that he soon lost all desire for salt while living among the Eskimos and subsisting on their diet. On the other hand vegetarians show a craving for salt. This is true of herbivorous animals as well as of man. Herbivorous animals are known to travel long distances to salt licks, or to drink of salt water. The necessity for salt by herbivorous animals has been explained as due to the loss of sodium chloride due

to the presence of potassium salts in the food. If the food contains large amounts of potassium salts the excretion of potassium will lead to a simultaneous loss of chlorine. It must be stated here, however, that this is as yet theoretical and has not been proved experimentally.

During starvation the elimination of chlorides is very slight as the body conserves its supply. After a month or more on a salt-free diet death occurs. Chloride is necessary to the animal for the production of the hydrochloric acid of the gastric juice. Sodium ions are necessary for the life of all animal cells. For example the heart will not beat if the sodium ion is not present in the perfusion liquid.

Chloride is usually estimated in urine or in deproteinized blood, by some form of the Volhard titration method. Silver nitrate is added in known excess and after filtration of the precipitated silver chloride, the excess of silver is titrated in an aliquot of the filtrate with standard ammonium thiocyanate. Ferric alum, which has been added as an indicator, gives a permanent brown color when the titration is complete.

Chapter VIII

THE BLOOD AND RESPIRATION

The Functions of the Blood

The blood is of service to the animal for the transportation of oxygen from the lungs to the tissues and for the transportation of carbon dioxide from the tissues to the lungs. The blood nourishes the tissues by bringing to them d. glucose, amino acids, fats and other lipins such as cholesterol and lecithin. The blood transports to the kidneys such waste products as urea, uric acid, creatinin, detoxication compounds, inorganic salts, water and many other substances. The blood is of importance for the transportation from one organ to another of hormones, such as secretin, adrenalin, thyroxin and insulin. Finally the blood transports heat from one part of the body to another and thus helps to keep the body temperature uniform.

The blood has the function of defending the organism against invading bacteria both by the phagocytic action of its white cells and by means of its antibodies. The lymph, chyle, cerebrospinal fluid, transudates and exudates can be regarded as blood that has been freed largely from its form elements and partly from its dissolved proteins.

Composition of the Blood

The blood is composed of a liquid portion, the plasma, and a portion consisting of solid particles, or form elements. These particles consist of the erythrocytes, leucocytes, blood platelets and blood dust, or chylomicrons.

Erythrocytes.—The erythrocytes are biconcave disks, the diameter of which varies in different animals. In man the

diameter is about 7 μ. The number of red blood corpuscles, or erythrocytes, in the blood of man is from 5 to 6 millions per cubic millimeter, in woman from 4 to 4.5 millions. The apparent number of red blood corpuscles is increased by anhydremia and the actual number is increased by a continued existence at great elevations, or by any prolonged anoxemia. In anemia the number diminishes and in pernicious anemia it may decrease to 500,000 per cubic millimeter. The erythrocytes of birds, reptiles and fishes are nucleated, but in all of the mammals except the camel and llama they are not nucleated.

Hemolysis.—When a slight amount of distilled water is added to blood the erythrocytes swell and become ovoid. The addition of more water causes the red cells to disintegrate and their red coloring matter, or hemoglobin, passes into solution, leaving behind the framework, or stroma. When this happens we say that the blood has been laked or hemolysed. The action of distilled water is believed to be due to the lowering of the osmotic pressure of the blood. The addition to blood of concentrated salt solutions, which have high osmotic pressures, draws water from the corpuscles, causing them to shrink and to become crenated. A solution containing 0.9 per cent of sodium chloride possesses the same osmotic pressure as mammalian blood and causes neither swelling nor shrinking of the erythrocytes. It is therefore said to be isotonic.

Substances that lower the surface tension of water cause laking. Some of these substances are soaps, salts of the bile acids, saponins, ether, chloroform and the higher alcohols. Other substances that cause laking are alkalis, acids, ammonium salts, snake venoms and the hemolysins. The latter are of the nature of antibodies and are specific in their action.

Hemoglobin.—The chromoprotein, hemoglobin, constitutes the greater part of the red blood corpuscles. A

substance similar to hemoglobin is present in the voluntary muscles and in the heart. In the crustacea and mollusks the place of hemoglobin is taken by hemocyanin, which is faintly blue when oxidized and colorless when reduced. Hemocyanin contains copper and is less efficient than hemoglobin as a respiratory pigment. The function of hemoglobin is the transportation of oxygen from the lungs to the tissues. Hemoglobin is of importance also for the transportation of carbon dioxide and as a buffer, as will be explained farther on. When hemoglobin is exposed to oxygen it immediately forms oxyhemoglobin. According to Barcroft and Hill 1.85 calories are produced when 1 gram of hemoglobin is oxidized to oxyhemoglobin. The hemoglobin molecule is composed of the protein globin united with the side-chain hematin. Globin has been classified as a histone. Hematin contains iron and is said to possess the empirical formula: $C_{34}H_{34}N_4FeO_5$. The ability of hemoglobin to unite with oxygen is dependent upon the presence of an atom of iron in the hematin. The red color of hemoglobin and of hemoglobin derivatives is due to the hematin group. Human blood contains from 14 to 15 per cent of hemoglobin, which is present largely as oxyhemoglobin. Hemoglobin is coagulated by heat and is precipitated by the usual protein reagents with a few exceptions. The globin part of the molecule contains a variety of the amino acids and is especially rich in histidine.

Hemoglobin crystallizes rather easily, depending upon its source. That from the rat, horse, squirrel and guinea pig crystallizes very readily, while the hemoglobin of man is more difficult to crystallize. The difference probably depends upon the solubility, the varieties that are least soluble being the easiest to crystallize. The method of crystallization consists in washing blood corpuscles with saline and centrifugation. The corpuscles are then laked with ether, cooled and treated with small amounts of

alcohol. Reichert and Brown have crystallized the hemoglobin from more than 100 animal species. They believe that the crystal habit is a criterion of species and have observed that hemoglobin crystals from the mule are intermediate between those from the horse and those from the donkey. It is probable that each animal species has its own hemoglobin. Heidelberger and Landsteiner have recently shown by the precipitin test that hemoglobins are specific for the species.

The absorption spectrum of hemoglobin and its derivatives is distinctive and can be used for their detection. Oxyhemoglobin in dilute solution gives two absorption bands between the Fraunhofer C and D lines. As the concentration of the oxyhemoglobin is increased the two bands come nearer together and finally unite to form a single band. Oxyhemoglobin shows absorption also in the ultraviolet. Hemoglobin, or reduced hemoglobin, as it is sometimes called, gives one absorption band which is somewhat nearer the red end of the spectrum than the bands of oxyhemoglobin. Hemoglobin can be readily prepared from oxyhemoglobin by pumping out the oxygen by means of a vacuum pump, or by adding a reducing agent, such as ferrous ammonium tartrate (Stokes' reagent). Reduced hemoglobin has a more purplish color than oxyhemoglobin and is more difficult to crystallize on account of its greater solubility.

Carbon Monoxide Hemoglobin.—Carbon monoxide unites with hemoglobin more readily than oxygen does and consequently when blood is exposed to an atmosphere containing only small quantities of carbon monoxide a considerable amount of carbon monoxide hemoglobin is produced. If an animal is allowed to breathe air containing 0.05 per cent of carbon monoxide 50 per cent of the animal's hemoglobin will be converted to carbon monoxide hemoglobin. This reaction is reversible and the carbon monoxide can be

removed by allowing the animal to breathe pure air, or oxygen. Unlike hemoglobin, carbon monoxide hemoglobin is not decomposed by the addition of alkali, but retains its bright red color. Its absorption spectrum differs but slightly from that of oxyhemoglobin.

Methemoglobin.—When blood is dried, or allowed to stand, the oxyhemoglobin slowly changes to methemoglobin, which is brown in color. The addition to blood of an oxidizing agent, such as potassium chlorate or potassium ferricyanide, immediately converts all of the oxyhemoglobin to methemoglobin. When the latter reagent is used in excess the oxygen combined with the oxyhemoglobin is given off quantitatively. The reaction has been used by Haldane for the estimation of oxyhemoglobin.

It was formerly believed that methemoglobin differed from oxyhemoglobin only in respect to the manner of the linkage of the oxygen. In 1910 Küster pointed out that oxyhemoglobin and reduced hemoglobin are both ferrous compounds, while methemoglobin is a ferric compound. Conant and Conant and Fieser have followed the reduction of methemoglobin by various reducing agents electrometrically and find that this is so. They propose the following schematic representation of hemoglobin, oxyhemoglobin and methemoglobin:

hemoglobin	Na_3Hb
oxyhemoglobin	Na_3HbO_2
methemoglobin	Na_2MHb

They have found that one hydrogen equivalent is involved in the change from methemoglobin to hemoglobin, as is also the case when potassium ferricyanide is reduced to potassium ferrocyanide. The number of sodium atoms in the formulas given above does not represent the actual number that occurs in the molecule.

Derivatives of Hemoglobin.—When oxyhemoglobin is

heated with dilute acid hematin is split off. Hematin is converted by reduction to hemochromogen, which occurs as a side-chain in reduced hemoglobin. When dried blood is heated with sodium chloride and glacial acetic acid the hematin is changed to hemin, which crystallizes readily as minute chocolate-colored crystals. This fact is taken advantage of for the identification of blood in old stains. Hemin is believed to be formed from hematin by the replacement of a hydroxyl group by a chlorine atom.

When hematin, or hemoglobin, is heated with rather concentrated sulphuric acid the iron is split off and hematoporphyrin is produced. This compound is found in traces in the urine. It has a peculiar effect when injected into white rats, making them extremely sensitive to the action of light. Hematin and its derivatives contain pyrrol residues. It is of interest to note that pyrrol groups are present also in the plant pigment chlorophyll.

Tests for Blood.—When blood is warmed with dilute acid and then shaken with ether, acid hematin is formed and dissolves in the ether layer. The absorption spectrum of this ethereal solution shows a distinctive band in the red. This offers a good means of detecting blood when it is present in moderate amount in the gastric or intestinal contents. Other tests for occult blood, a term that signifies blood that has been made unrecognizable by laking and mixture with other materials, depend upon the fact that the hematin part of the hemoglobin molecule can act as a carrier of oxygen. Certain substances, such as guaiaconic acid, phenolphthalin (reduced phenolphthalein), benzidine and aloin, are oxidized by hydrogen peroxide to form highly colored products, provided that a catalyst, or oxygen carrier, is simultaneously present. In the absence of a carrier no oxidation occurs. As the oxydases and peroxydases are carriers of oxygen it is necessary to boil the material to be tested in order to inactivate these enzymes. The guaiac

(guaiaconic acid) test is the least sensitive of those mentioned above. The phenolphthalin test is capable of detecting 1 part of blood in 65 million parts of water.

The Estimation of Hemoglobin.—The amount of hemoglobin in blood can be determined by saturating the blood with oxygen at atmospheric tension and then estimating by volume the oxygen that is liberated when the blood is mixed with an excess of potassium ferricyanide. Ammonia must be added to the blood to prevent the escape of carbon dioxide. The ammonia also causes laking of the corpuscles. This aids in releasing the oxygen. Normal male blood that has been completely oxidized gives off 18.5 cc. of oxygen from every 100 cc. The determination can be made with the differential apparatus of Haldane, or the apparatus of Van Slyke can be used. In the Van Slyke method the extraction of oxygen is aided by subjecting the blood to a Torricellian vacuum. Van Slyke advises the use of saponin to hasten the laking of the erythrocytes.

There exist many colorimetric methods for the estimation of hemoglobin. In these methods the blood is usually laked with water and the hemoglobin is converted to acid hematin, or carbon monoxide hemoglobin, and compared with a standard. Whatever standard is used must be checked up by one of the gasometric methods.

Leucocytes.—Normal human blood contains from 7500 to 15000 leucocytes, or white cells, per cubic millimeter. The number increases somewhat after the ingestion of food. In infections the number is considerably increased, although there may be a decrease in the first stages of the disease. In leucemia the number of white cells becomes very great. Six kinds of leucocytes are found in the blood. The most numerous are the polymorphonuclear neutrophiles, which constitute 70 per cent of the total. The small lymphocytes constitute 20 per cent and the remainder consists of the

large lymphocytes, transitional leucocytes, eosinophiles and basophiles.

The specific gravity of the leucocytes is less than that of the red blood corpuscles. Hence, when blood clots slowly, or when oxalated blood is centrifuged, the leucocytes settle out on top of the layer of erythrocytes. The leucocytes possess the property of ameboid movement and contain such enzymes as erepsin and peroxydase. They contain albumins, globulins and nucleoproteins.

Platelets.—The platelets can be obtained from oxalated blood by centrifuging off the corpuscles at moderate speed and then centrifuging the plasma that has been obtained at a very high rate of speed. The platelets are possibly composed of protein loosely combined with cephalin. They are believed to play an important part in the coagulation of blood, as will be described later on.

Chylomicrons.—The chylomicrons, or hemoconia, are composed in part, at least, of fat and other lipins. These particles are extremely small and when observed under the dark-field microscope can be seen to be in rapid Brownian movement. The number of chylomicrons is increased greatly during the absorption of fat, which is transported in this form from the intestine to places of fat deposit.

Blood Plasma.—Blood plasma is a complex solution of proteins, of organic compounds such as urea, glucose, uric acid, etc., of inorganic salts and of a variety of other substances about which little is known. The plasma of mammals contains from 7 to 9 per cent of proteins. These are fibrinogen, two serum albumins, two or more serum globulins and a nucleoprotein. Fibrinogen is a globulin and is the compound involved in the process of blood coagulation. In this process fibrinogen is changed to fibrin, passing from a sol to a gel. Fibrin is insoluble in water or salt solutions, but dissolves readily in dilute alkali. Fibrinogen is thought to be synthesized in the liver, inasmuch as the blood fibrin-

ogen disappears after extirpation of the liver and after the liver has been damaged by phosphorus poisoning. Fibrinogen is the most readily precipitated of the globulins and can be entirely salted out of solution by saturation with sodium chloride. Methods for the preparation of fibrinogen have been described by Hammarsten, McLean, and Dale and Walpole.

There exist two serum albumins. One of these can be crystallized, the other has not been crystallized. At least two serum globulins exist. According to Hofmeister and Pick, euglobulin is insoluble in distilled water and is precipitated by adding 28 to 36 volumes per cent of saturated ammonium sulphate. Pseudoglobulin is so called because it is soluble in distilled water. It can be salted out by adding 36 to 44 volumes per cent of saturated ammonium sulphate. The antibodies that are produced in the development of immunity appear to be associated with the serum globulins. In many infectious diseases the globulin fraction of the blood is increased. In this connection the recent discovery that the enzyme urease is a globulin is of interest.

The blood contains small quantities of a large number of organic compounds of relatively simple structure. Among these are d. glucose, lactic acid, urea, urates, acetone bodies, amino acids, creatin, creatinin and the recently discovered thiasine. Traces of maltose may be present. The blood contains neutral fats, free cholesterol, cholesterol waxes, lecithin and probably cephalin. These substances are not in true solution but are associated either with the form elements of the blood, or with the proteins. Blood contains a large amount of the enzyme catalase and small amounts of the enzymes amylase and lipase. Hormones are doubtless present in minute amounts. The gases present in blood are nitrogen, oxygen, carbon dioxide and argon. Small amounts of hydrogen and marsh gas may be present in the

blood of herbivora, in whom a considerable intestinal fermentation occurs normally.

The inorganic constituents of blood are composed of the cations sodium, potassium, calcium and magnesium and the anions chloride, bicarbonate, phosphate and sulphate. Sodium chloride is present in a concentration of from 0.5 to 0.6 per cent. It has been stated by some authors that the erythrocytes contain slightly less glucose, urea and chlorides than the plasma. This may be due to the fact that there is less liquid present in the red cells than in the plasma. The erythrocytes, on the contrary, are said to contain more amino compounds than the plasma, more inorganic phosphorus and all of the thiasine. Here is evidence of either an adsorption or of a selective solubility.

Physical Constants of Blood.—Figures given in the literature for the percentage by weight of blood in the human body range from 5 to 8 per cent, the variation being due largely to the inaccuracies of the methods that have been used. The freezing point of human blood is −0.56° C. and that of other mammals approaches this value rather closely. This freezing point depression corresponds to an osmotic pressure of about 8 atmospheres. The specific gravity is between 1.045 and 1.075 and is greater in males than in females. The pH is 7.35.

The Coagulation of Blood

One of the most important of the properties of blood is its ability to coagulate spontaneously after it escapes from the blood vessels. Human blood clots from 5 to 10 minutes after shedding. The importance of this property of blood is made manifest in the disease known as hemophilia, where for some unknown reason the blood does not clot and death may be caused by excessive loss of blood from minute wounds.

The process of blood coagulation consists in a transfor-

mation of the soluble fibrinogen to the insoluble fibrin. The fibrin separates in strands of very minute crystals, entraining the corpuscles and forming a clot, or gel. On standing the clot contracts, squeezing out some of the liquid, which is known as serum. The contraction of a clot is known as "syneresis." It occurs after the clotting of milk and after the clotting of vegetable pectins.

The formation of fibrin from fibrinogen is believed to be brought about by a body known as thrombin, or fibrin ferment. Thrombin is not present in circulating blood, but is formed during coagulation. It is present in considerable amount in the serum and in fibrin. It can be conveniently prepared from washed and minced fibrin by extraction with 8 per cent sodium chloride at a low temperature. Thrombin is somewhat unstable and loses its activity upon standing for some time at room temperature. Although Alexander Schmidt advanced the idea that thrombin is an enzyme it appears to act in a quantitative manner and to become used up in the process of coagulation. According to definition an enzyme is not used up in the catalysis of a reaction and a very small quantity of enzyme can facilitate the decomposition of an extremely large amount of substrate. Furthermore, thrombin that has been freed from salts withstands the temperature of boiling water, while enzymes are inactivated by this treatment.

According to the theory of Moravitz, the blood contains the zymogen prothrombin, or serozyme, which is changed into thrombin by the combined action of calcium ions and the substance thrombokinase. Thrombokinase is known also as thromboplastin and as cytozyme. Although circulating blood contains all of the elements necessary for coagulation it does not ordinarily clot in the vessels, possibly because no thrombokinase is liberated. After blood is shed thrombokinase is set free and clotting occurs. The blood platelets are particularly rich in the phosphatide

cephalin and this body has been identified by Howell as thrombokinase. When blood comes in contact with lacerated tissues its clotting is hastened. Here the effect is probably due to the cephalin of the tissues. Blood that is allowed to flow from a vein or artery through a paraffined cannula directly into a paraffined beaker does not coagulate for a long time. We can explain this phenomenon by assuming that materials that the blood does not wet do not cause the platelets to disintegrate and give off their cephalin. On the contrary, the addition to blood of dust, sand, or any matter that blood wets will hasten the time of coagulation. The effect of cephalin is very potent. E. Zunz and his collaborators found that solutions of impure cephalin after dilution with 200,000,000 volumes of water would still cause the formation of a clot if added to purified solutions of fibrinogen that contained prothrombin and calcium chloride. Lecithin is said to hasten the coagulation of fibrinogen provided that some cephalin is present, but to be incapable of taking the place of cephalin. Lecithin is accordingly called a thromboplastic substance.

Although calcium ions are necessary for the coagulation of blood, as Arthus and Pagès first showed, the calcium is necessary only for the formation of thrombin and is not required for the precipitation of fibrin once thrombin has been produced. Accordingly potassium oxalate is usually employed for preventing the clotting of blood, as it precipitates the calcium as the insoluble oxalate. Folin and Wu advise the addition of 20 mg. of potassium oxalate to each 10 cc. of blood in their system of blood analysis. The addition to 100 parts of blood of 0.3 parts by weight of sodium citrate prevents coagulation. Citrate exerts its effect not by precipitating the calcium, but by depressing the concentration of calcium ions. Fluorides prevent clotting in a manner analogous to that of oxalates. The clotting of blood is retarded by cooling and is accelerated, within

limits, by heat. The addition to blood of a sufficient amount of the substance hirudin, which can be obtained from the salivary glands of the leech, prevents coagulation. Concentrated salt solutions prevent coagulation by precipitating the fibrinogen. When peptone is injected into the circulation the blood subsequently loses its ability to clot.

Woolridge showed that extracts of the lungs, and to a less extent extracts of other organs, have a powerful effect upon blood coagulation and will even cause intravascular clotting if injected intravenously. He has called this material "tissue fibrinogen." Mills has shown that if small amounts of the lung extract are given by mouth the coagulation time of the subject's blood is considerably shortened and that if small amounts of the lung extract are injected into the circulation the blood loses its power of clotting. Cephalin suspensions do not cause intravascular clotting when given intravenously, from which it appears that the active material of lung extracts must consist of something other than cephalin.

The problem of the coagulation of the blood is complex and difficult. The known facts comprise so much that has not been, or that cannot be explained on a chemical basis that the most generally accepted theories of blood coagulation make little appeal to the biological chemist.

The Clinical Analysis of Blood

Within the past ten years there has been developed a number of methods for the analysis of blood for diagnostic purposes. These methods are of value also in following the progress of a disease and the effect of treatment and in deciding when it is unsafe to perform surgical operations. The methods require only small amounts of blood, are usually colorimetric and require very little time to perform. Nearly all of them have been developed in America. Some

of the more prominent workers in this field are Folin, Benedict, Van Slyke, Myers and Bloor.

The values that are most frequently determined are for sugar, total non-protein nitrogen, urea nitrogen, creatinin, uric acid and the carbon dioxide combining power of blood plasma. The first five of these can be determined by the Folin-Wu system of blood analysis. This has the advantage that all five analyses are made upon one sample of deproteinized blood. The methods will not be described here, as the student can find them in any laboratory manual of biological chemistry. The table below gives in the first column the normal values of a number of the constituents of the blood and in the second column mention is made of the variations encountered in disease.

	normal value	variation in disease
	per 100 cc. of blood	
	mg.	
total non-protein nitrogen	25–35	30–50 in mild nephritis to 400 in acute nephritis
urea nitrogen	9–19	to 30 in mild nephritis to 300 in acute nephritis
sugar	80–110	to 140 to 600 in diabetes
creatinin	1–2	to 4 in mild nephritis to 35 in acute nephritis
creatin	4–5	increased in nephritis
uric acid	1–3	increased in gout, arthritis and nephritis
amino acid nitrogen	6–8	
undetermined nitrogen	10–17	
ammonia nitrogen	<0.1	
fat	650	increased in lipemia
lecithin	325	increased in lipemia
cholesterol	140	increased in lipemia
sodium chloride	500–600	increased in nephritis
calcium	10	decreased in rickets
inorganic phosphorus	3.7	decreased in rickets

RESPIRATION

The Transport of Oxygen

The ability of the blood to transport oxygen and carbon dioxide is shown by the analysis of inspired and expired air, as given in the following table:

	inspired air vols. per cent	expired air vols. per cent
oxygen	20.96	16
carbon dioxide	0.04	4.4
nitrogen	79.0	79.6

In the lungs the hemoglobin of the blood becomes almost completely saturated with oxygen. At the same time the blood gives up a considerable portion of its carbon dioxide. In the tissues the process is reversed. The passage of oxygen from the alveoli of the lungs across the lung epithelium is believed by Haldane not to be entirely a simple diffusion, but to involve a secretory action of the epithelial cells. However, direct proof of this assumption is lacking. The reaction of hemoglobin with oxygen to form oxyhemoglobin is not physical in nature, but chemical. It is as follows:

$$Hb + O_2 \rightleftarrows HbO_2$$

The reaction is reversible and the point of equilibrium depends upon the concentration of oxygen, or upon the partial pressure of oxygen, which amounts to the same thing. According to the mass law:

$$\frac{[HbO_2]}{[Hb] \times [O_2]} = K$$

If the degree of saturation of a pure solution of hemoglobin with oxygen is plotted against the oxygen tension in milli-

meters of mercury pressure one obtains a curve that is a right-angled hyperbola. The curve shows that when the oxygen tension is not less than 70 per cent of the tension at which oxygen occurs in our atmosphere, between 90 and 100 per cent of the hemoglobin will exist as oxyhemoglobin. With oxygen tensions less than this the saturation of hemoglobin with oxygen falls off very rapidly. This factor is of advantage in allowing the tissues to remove oxygen from hemoglobin.

The dissociation curve of oxyhemoglobin is affected by temperature, by acids and by inorganic salts. With increase in temperature hemoglobin unites with less oxygen. The addition of acid has a similar effect. Bohr was the first to show that hemoglobin takes up less oxygen with increasing tensions of carbon dioxide. It can be seen that this effect is of advantage in the tissues where the blood gases are exchanged, as the carbon dioxide entering the blood drives out the oxygen. The dissociation curve of hemoglobin as its exists in blood differs from the curve given by purified hemoglobin in that that of the former is slightly S shaped. This is believed to be due to the action of the inorganic salts of the blood upon the aggregation of the hemoglobin molecules.

The Transport of Carbon Dioxide

The carbon dioxide content of normal arterial blood is about 50 volumes per 100 cc., while that of venous blood is from 55 to 60 volumes. Inasmuch as the amount of carbon dioxide that will dissolve in 100 cc. of pure water at body temperature and at the tension of carbon dioxide which prevails in the tissues is only about 10 cc., it is apparent that the blood must possess some means of combining with this gas. When carbon dioxide enters the blood from the tissues most of it unites with the blood alkali and is transported as alkali bicarbonate. Some free carbon

dioxide is also present in blood. The proportion of free carbon dioxide to sodium bicarbonate is about one to twenty. The alkali which is furnished by the blood for the transport of carbon dioxide comes largely from the hemoglobin. Van Slyke claims that from 80 to 95 per cent is supplied from this source. When the blood reaches the lungs the carbon dioxide escapes and the alkali again unites with the hemoglobin to form a salt. The sodium chloride in the plasma plays an important rôle in this process. When carbon dioxide enters the blood its reacts with the sodium ions of the plasma to form sodium bicarbonate. The chlorine ions, together with hydrogen ions coming from the carbonic acid, immediately enter the erythrocytes and there unite with alkali furnished by the hemoglobin. In this manner hemoglobin is enabled to furnish alkali for the transportation of carbon dioxide without leaving the corpuscles. Some carbonic acid doubtless enters the corpuscles and unites directly with alkali furnished by the hemoglobin. The sodium and potassium ions are not exchanged between the plasma and the corpuscles according to recent work by Doisy and Eaton.

It has been found that reduced hemoglobin is a weaker acid than oxyhemoglobin. This difference in acid strength makes easier the liberation of alkali that occurs simultaneously with the reduction of oxyhemoglobin to hemoglobin. The alkali phosphates, which are present largely in the erythrocytes, furnish some alkali for the transportation of carbon dioxide. Alkali is also supplied by the plasma proteins.

The Buffer Action of the Blood

One of the most remarkable properties of the blood is its ability to combine with the carbon dioxide that is given off by the tissues without changing appreciably in reaction. This is due to the presence in blood of substances that have

been called "buffers." A buffer is a substance that keeps the reaction of a solution from changing to any considerable extent when acid or alkali is added. The significance of buffers was first pointed out by L. J. Henderson and by Sörenson. As an illustration of a buffer we can take a solution containing both primary and secondary sodium phosphates. If we add hydrochloric acid to this solution the acid will react with the secondary sodium phosphate and no considerable change will occur in the hydrogen ion concentration until nearly all of the secondary phosphate has been used up. The reaction is:

$$HCl+Na_2HPO_4=NaCl+NaH_2PO_4$$

If we add sodium hydroxide to our phosphate buffer the reaction is now between the alkali and the primary sodium phosphate as follows:

$$NaOH+NaH_2PO_4=H_2O+Na_2HPO_4$$

Primary phosphates are weak acids, while secondary phosphates are weak bases. In a mixture of both the acid dissociation of one and the alkaline dissociation of the other will be depressed. Hence all mixtures of these phosphates are nearly neutral.

The buffers of the blood are the proteins (which exist in part as alkali salts), carbonic acid and bicarbonates, and phosphates. A buffer is most effective when the concentration of the acid component is equal to the concentration of the basic component. For carbonic acid and sodium bicarbonate this occurs when the pH is 6.1. For phosphates it occurs when the pH is 6.8. As the reaction of the blood is 7.35, it can be seen that neither of these buffers is at its most effective point when in the blood. The most effective point for oxyhemoglobin is when the pH is 7.16 and for reduced hemoglobin is when the pH is 7.3. Hence reduced hemoglobin, which receives carbonic acid from the tissues,

is the most perfect of the buffers of the blood. The animal has taken the carbonic-acid-bicarbonate buffer and the phosphates buffer as it found them, but hemoglobin can be considered to be a product of chemical evolution. The change in acid strength that takes place when oxyhemoglobin is reduced to hemoglobin is yet another aid in preventing the reaction of the blood from changing when it takes up carbon dioxide. In connection with the subject of "buffers" the student is asked to consult the material given on this topic in the chapter dealing with the physical chemistry of protoplasm.

Chapter IX

PHYSIOLOGICAL DETOXICATIONS

The animal is often faced with the necessity of dealing with substances of a more or less poisonous nature that are produced as a result of the action of bacteria, or that happen to be present in the food.

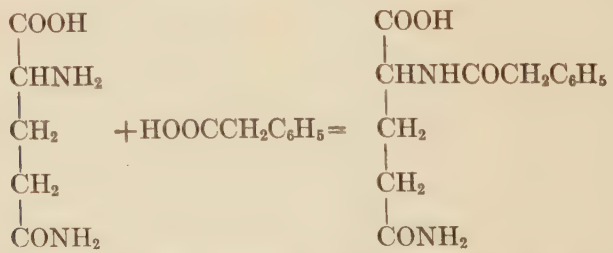
When the poisonous substance is of protein nature the animal reacts by producing an antibody and under favorable conditions may become immune. In case the poisonous substance is not a protein, antibodies are not produced and immunity is not acquired as the result of the administration of the poison. Nevertheless animals have been shown to be capable of acquiring what is known as tolerance towards certain toxic substances of this class. The ability to acquire a tolerance for nicotine, arsenic, alcohol, morphine and cocaine is well known. In certain instances this tolerance may be due to an increased power of detoxicating the harmful substance, in other cases there may be a decreased reaction on the part of the tissues, or a decrease in the rate of absorption from the gastro-intestinal tract. The ability of the cells to protect themselves against poisons is remarkable, and unless the amount of the poisonous substance is so great that the cells are overwhelmed, the process of recovery is usually prompt.

The mode of defence depends upon the nature of the poison and also upon the species of the animal. The substance is destroyed by oxidation if possible. If oxidation is not possible the poisonous material is usually conjugated with some compound that contains carboxyl or hydroxyl

groups, and in this manner is converted into a product that is much more soluble in water than the original compound. In this manner the ability of the poison to dissolve in the nervous system is removed and the detoxication product remains in solution in the blood stream and is soon excreted by the kidneys.

In the discussion of the hypothesis of beta oxidation the ability of the dog to detoxicate benzoic acid by conjugating it with glycocoll to form hippuric acid has already been mentioned. The production of hippuric acid has been shown to occur in most mammals, but not in birds. The glycocoll used in this process is believed to be produced from urea, since the animal does not possess a store of glycocoll and since the administration of benzoic acid causes a diminution in the excretion of urea. When very large amounts of benzoic acid are fed there occurs an increased destruction of protein.

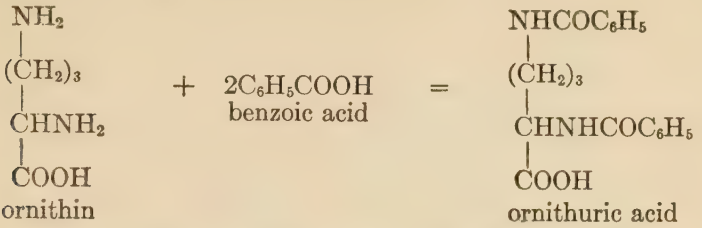
In the dog phenyl acetic acid also is conjugated with glycocoll with the formation of phenaceturic acid. This does not happen in man. Thierfelder and Sherwin have shown that when phenyl acetic acid is fed to the human subject it is conjugated with glutamine to form phenacetyl glutamine, or glutamuric acid:



glutamine phenylacetic acid glutamuric acid

In birds benzoic acid and phenyl acetic acid are detoxicated by conjugation with ornithin. This type of detoxi-

cation is the most important, if not the only one in birds. The reaction with benzoic acid is:



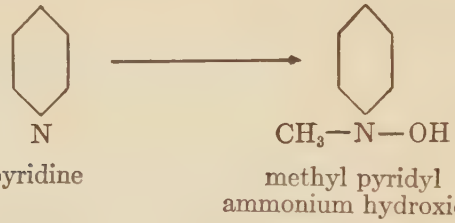
A great variety of compounds is detoxicated by conjugation with glycuronic acid. Some of these substances are: camphor, menthol, chloral hydrate (after reduction), resorcin, curare, strychnine, and the like. When alcohols are united with glucuronic acid an ether is formed and a stable glycuronate results. Acids combine with glycuronic acid to form esters. The ester type of glycuronate is readily hydrolysed and consequently has a reducing action on reagents used for the detection of sugar in urine. Glycuronates are often formed with the same compounds that are detoxicated with sulphuric acid, especially if the poisonous compound is present in large amount.

The phenols, cresols, indol (after oxidation to indoxyl) and skatol (after oxidation to skatoxyl) are usually detoxicated by conjugation with sulphuric acid and appear in the urine as ethereal sulphates. The process of detoxication usually does not proceed to completion and some of the phenol is found in the urine in the free state.

Demethylation, or the removal of methyl groups, takes place after feeding certain plant purines to animals. Caffeine, theobromine and theophylline are detoxicated in this manner, as has been discussed in the chapter on the purines. The products thus formed are eliminated in the urine. It has been found that different animal species demethylate differently.

Methylation, or the addition of methyl groups, is a rare

reaction. It has been found to occur when pyridine is fed to certain animals. The detoxication product is methyl pyridyl ammonium hydroxide and the change may be illustrated as follows:

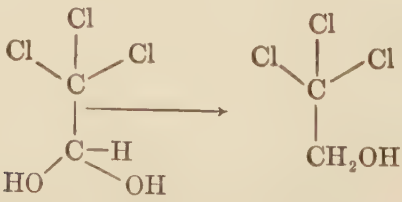


pyridine — methyl pyridyl ammonium hydroxide

In some instances a substance undergoes a preliminary oxidation before conjugation with another compound. Thus indol is oxidized to indoxyl before conjugation with sulphuric acid, and skatol is oxidized to skatoxyl:

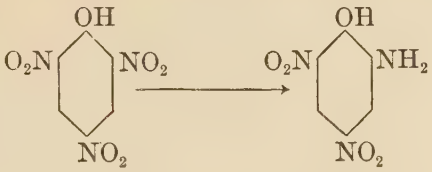
indol — indoxyl — skatol — skatoxyl

Reduction is sometimes employed in dealing with poisonous substances. It will be recalled that acetoacetic acid is partly reduced to beta-oxybutyric acid by the diabetic animal. Chloral hydrate is reduced to trichlor ethyl alcohol by the animal before it is conjugated with glycuronic acid, the reaction being as follows:



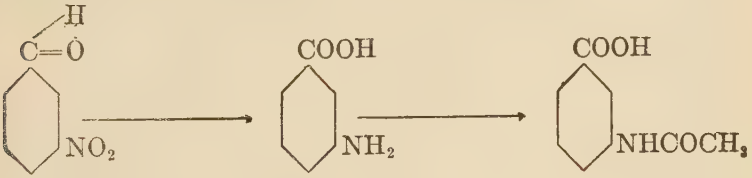
chloral hydrate — trichlor ethyl alcohol

In certain cases nitro compounds are reduced to amino compounds. Picric acid, for example, is said to be reduced to picramic acid thus:



picric acid picramic acid

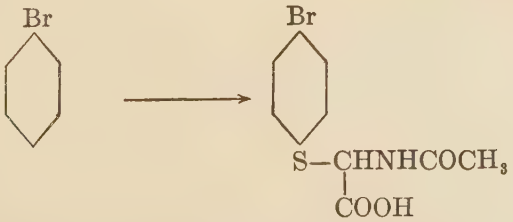
Again, when meta nitro-benzaldehyde is fed to rabbits it is subjected to a process of simultaneous oxidation and reduction, meta amino-benzaldehyde being formed and then acetylated, as shown by the following:



meta nitro-benzaldehyde meta amino-benzoic acid meta acetyl-amino-benzoic acid

When ortho nitro-benzaldehyde is ingested by man it is oxidized to ortho nitro-benzoic acid and excreted as such.

If the poisonous mono brom-benzene is fed to dogs it is detoxicated by conjugation with cystein, followed by acetylation. The ultimate product is para brom-phenyl-mercapturic acid:



This reaction may occur in man, but it has not yet been demonstrated.

Chapter X

ENZYMES

Balanced Reactions

Many of the chemical reactions of plants and animals belong to the type known as balanced reactions. The balanced reaction is one that proceeds slowly to a certain point characteristic of the reaction and known as the point of equilibrium. The reaction then ceases, without having continued to completion. A discussion of the balanced reaction between ethyl alcohol and acetic acid, together with an application of the mass law, can be found on page 238. In the organism the balanced reaction is brought to completion because the products of the reaction are removed as fast as they are formed. The digestion of fat may be cited as an example. As fast as fatty acids and glycerol are produced in the intestine by the action of the enzyme steapsin, these products are absorbed by the intestinal epithelium. Thus, the reaction continues to completion and the point of equilibrium is never reached.

Catalysis

Catalysts.—Balanced reactions are, par excellence, the sort of reactions that are capable of acceleration by catalysts. A catalyst, as defined by Ostwald, is a substance, or compound, that increases the speed of a chemical reaction without being used up in the process, or becoming a part of the product formed. Catalysts have been found to be of enormous importance in the field of chemical industry. In the contact process for the manufacture of sulphuric acid, platinized asbestos is required for the oxidation of

sulphur dioxide to sulphur trioxide by atmospheric oxygen. In the commercial hydrogenation of fats, finely divided nickel is the catalyst used to facilitate the reaction between gaseous hydrogen and the unsaturated fatty acids. The new German process for the synthesis of methanol from carbon monoxide and hydrogen is dependent upon the catalytic action of zinc oxide containing traces of chromium, uranium, or vanadium.

The catalyst does not alter the point of equilibrium of a reaction, but is effective on account of its ability to increase the rate. Its action can be compared somewhat crudely, to the effect of oil upon a piece of machinery. It is possible to have what is known as negative catalysis, where a reaction is hindered, instead of being hastened. A well known example of negative catalysis is the effect of ether vapor in preventing the oxidation of yellow phosphorus by atmospheric oxygen.

Enzymes as Catalysts.—The cell uses catalysts of a special sort to hasten the speed of certain reactions and to make reactions proceed in a certain direction, where side reactions are possible. These catalysts are called enzymes, a word meaning "in yeast." Under the influence of enzymes, various hydrolytic, dehydrolytic, oxidative and reductive reactions proceed in plant and animal cells at relatively low temperatures and at relatively rapid rates. Fats, proteins and carbohydrates are hydrolysed in the digestive tract and, after absorption, the products are resynthesized to fat, glycogen, or tissue protein. The synthetic reactions are presumed to be catalysed by enzymes similar to those that cause the hydrolysis. Oxidizing enzymes transform the purine bases into uric acid and oxidize various phenols; deaminizing enzymes remove amino groups from amino acids, and carboxylases decompose carboxyl groups.

It is still an open question whether or not the oxidation of glucose and fat is of an enzymic nature. Oxidizing

enzymes acting on sugar and fat have never been demonstrated, and it is necessary to assume, either that enzymes of this sort cannot exist independently of the living cell, or else that these important oxidative reactions are catalysed by glutathion, or by some similar substance, or system of substances. In its reduced form glutathion is a dipeptide composed of cystein and glutamic acid, and cannot be included among the enzymes as it is a crystalloid and is thermostable. Other thermostable catalysts are known to exist in animals. Jones has demonstrated in the pancreas of the pig a thermostable catlyst which hydrolyses nucleic acid to mono-nucleotides. Hammett has shown that muscle contains a thermostable substance which transforms creatin to creatinin.

Enzymes differ from catalysts in general in being the products of plants and animals, in possessing colloidal properties, and in being readily destroyed by heat. Many enzymes, when in the moist state, are inactivated at a temperature of from 60° to 80° C. and all enzymes are destroyed by boiling. In the dry state enzymes withstand temperatures considerably higher than 100° C.

Terminology of Enzymes

Substrate Classes.—Enzymes are usually classified according to the substrate, or substance upon which they act. Fat-splitting enzymes are known as lipases, starch-digesting enzymes are called amylases—after the Latin for starch—enzymes that hydrolyse proteins are called proteases.

The enzymes known as oxydases have been named on the basis of their action, rather than on the basis of the substrate. This is because of the fact that the oxydases comprise a heterogeneous group and the individual members act on a variety of substances.

Many enzymes have been given individual names in order to distinguish them from other members of the same

group. As examples of individual amylases, we have ptyalin of saliva, amylopsin of pancreatic juice and malt diastase in malt. Some of the proteases, or proteoclastic enzymes, are pepsin, trypsin and erepsin of the digestive tract, bromelin in pineapple rind and papain in the pawpaw.

Endo- and Exo-enzymes.—Enzymes were formerly divided into two groups, organized and unorganized. The organized enzymes were those incapable of action without the presence of living cells. Unorganized enzymes, such as ptyalin, pepsin, trypsin and the amylase of malt could exist and exert their action in the absence of living cells. In 1897 Buchner broke down the distinction between organized and unorganized enzymes by showing that zymase, the organized enzyme of yeast, could be prepared entirely free from cellular material and that it would still bring about the alcoholic fermentation of sugar. Buchner prepared zymase solutions by grinding brewer's yeast with infusorial earth. The grinding process ruptured the yeast cells and made it possible to squeeze out the cell contents by means of a powerful press. Later Buchner was able to obtain a cell-free enzyme from acetic acid bacteria that fermented alcohol with the production of acetic acid. Today we do not speak of organized and unorganized enzymes, but we use the term endo-enzyme with reference to an enzyme that is not secreted by the cell, and we call the enzyme that is secreted by the cell an exo-enzyme, or extracellular enzyme.

The Influence of Temperature

The influence of temperature upon reactions catalysed by enzymes is two-fold. Increase in temperature causes an increase in the rate of the reaction up to a certain point, which is known as the optimum temperature. The rate of increase in the velocity of reaction is doubled, or trebled for each rise in temperature of 10° C. After the optimum

point is reached, further increase in temperature has the effect of slowing the speed of reaction. This is due to the destructive action of heat upon the enzyme. The optimum temperature represents a mean between the destructive and the accelerative actions of heat. Animal enzymes have been elaborated for work at the temperature of the animal body and their optima are usually at about 40° C. Plant enzymes have optimal temperatures that are considerably higher than this. The optimum temperature for the action of urease is between 50° and 55° C. For malt diastase the optimum is still higher.

Changes in temperature alter the equilibrium points of balanced reactions. This effect is of importance in connection with catalysts, such as spongy platinum, which are active over wide ranges of temperature; but in the case of enzymes which are practically inactive at 0° C. and which are destroyed by boiling, the possible shift in the point of equilibrium is a negligible quantity. Moreover, temperature change has the least effect upon reactions in which the exchange of energy is small. Most of the reactions that are catalysed by enzymes involve so little exchange of energy that it is a very difficult matter to measure their heats of reaction.

The Nature of Enzymes

Practically nothing is known about the chemical nature of enzymes. In many cases enzyme preparations that have been subjected to methods of concentration and purification have been shown to be largely, or completely composed of protein. In other cases enzyme preparations have been obtained free from protein. There is no valid reason for supposing that all enzymes should belong to a single class of chemical compounds; it appears more likely that they are chemically diverse.

Physically, enzymes are hydrophile colloids and do not

diffuse through membranes. They are soluble in water, but are insoluble in strong alcohol, ether, chloroform and acetone. They appear to be optically active and of large molecular weight. On account of their unknown nature there is no way to test for their presence except through their action.

Tests for Enzymes.—Enzyme action can be followed either by observing the disappearance of the substrate, or by observing the production of reaction products. Besides using chemical tests, one can make use of such physical phenomena as changes in optical rotation, in the refractive index, in the viscosity and in the electrical conductivity. The presence of pepsin is demonstrated by showing that protein is digested to the proteose stage in the presence of dilute hydrochloric acid. If protein is digested in a neutral, or slightly alkaline medium, trypsin may be considered to be present. The hydrolysis of alpha glucosides is accepted as proof of the presence of maltase, while the hydrolysis of beta glucosides shows that we are dealing with the enzyme emulsin.

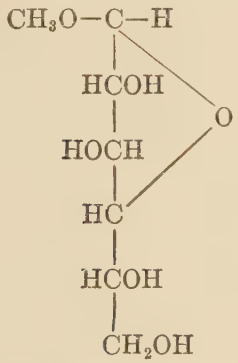
Owing to the catalytic nature of enzymes, extremely small amounts can be detected if sufficient time is allowed for their action. For example, extracts of the jack bean containing large amounts of protein and carbohydrate can be diluted with distilled water until protein and carbohydrate can no longer be detected by chemical test. The dilute solution can readily be shown to contain urease by its production of ammonium carbonate after the addition of a crystal of urea. Theoretically, a given quantity of enzyme should be able to catalyse the reaction of an indefinite amount of substrate, if allowed a sufficient interval of time in which to act. It has been shown in certain instances that small amounts of impure enzyme preparations can effect the decomposition of several hundred thousand times their weight of the material acted upon. Sher-

man, Caldwell and Adams prepared a pancreatic amylase that digested 4,000,000 times it weight of starch. However, enzymes are unstable substances and even under otherwise favorable conditions they are subject to a process of spontaneous decay when in solution or when moist. In the dry state enzymes are usually stable, provided that they have not been previously subjected to processes of purification. The author has kept a sample of jack bean meal in the laboratory for five years without being able to detect an appreciable decrease in its urease content.

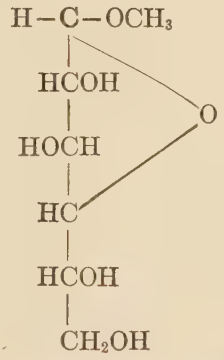
Specific Action of Enzymes.—One of the most interesting and remarkable of the properties of enzymes is their specific action. The ordinary catalysts are not specific and are usually capable of accelerating reactions of the most diverse kinds. Hydrochloric acid aids in the hydrolysis of all esters and platinum is an effective catlyst for an endless number of oxidations and reductions. The enzyme, on the contrary, has a special duty to perform and has been constructed by nature for the catalysis of one kind of reaction. The specific action of enzymes led Emil Fischer to compare the enzyme to a key which will fit only one lock. This property is of considerable value when it is necessary to identify certain chemical compounds. Thus, yeast, which contains the enzyme zymase, ferments d. glucose and d. fructose but does not affect the pentose sugars. Fermentation with yeast is of use in certain cases where it is difficult to decide whether the reducing power of a sample of urine is, or is not, due to the presence of d. glucose. The application by Marshall of the enzyme urease for the quantitative determination of urea in blood and urine marked a noteworthy advance in the ease, rapidity and accuracy of urea determinations. Urease decomposes no constituent of blood or urine but urea and with one exception is entirely without action on the substituted ureas. The enzymes sucrase, maltase, lactase and emulsin are of value for the

analysis and differential determination of disaccharides, when several are present in solution; in some cases the identification of a sugar is practically impossible with the ordinary reagents.

One of the most frequently cited instances of enzyme specificity is that of maltase and of emulsin. Maltase, which is widely distributed in plants and animals, hydrolyses alpha glucosides, but is without action on beta glucosides. Emulsin, which occurs in almonds and many other seeds, hydrolyses beta glucosides, but not alpha glucosides. The difference between the alpha and the beta forms of individual glucosides is stereoisomeric, or due to a difference of arrangement in space. This is illustrated by the structural formulas for the alpha and beta methyl glucosides, which are given below:



alpha methyl glucoside



beta methyl glucoside

It seems likely that in order to exert its hydrolytic effect the enzyme must make contact with the glucoside at more than one point. Maltase is unable to hydrolyse beta glucosides because its arrangement in space prevents it from making a good contact. The same thing is true with respect to the configurations of emulsin and the alpha glucosides. Inasmuch as many kinds of alpha glucosides are hydrolysed by maltase and many kinds of beta glucosides are hydro-

lysed by emulsin, it is evident that the enzymes do not come in contact with the radicals that are united with the glucose molecule.

In the discussion of polypeptides (Chapter IV) it was stated that those polypeptides which contain unnatural isomers of the amino acids are not attacked by trypsin, erepsin and the tissue erepsins. The inability of enzymes to act upon stereoisomers that do not occur in nature has been illustrated with a large number of different substances, by the use of enzyme preparations, by feeding the compounds in question to animals, or by adding them to bacterial cultures.

The specificity of enzymes explains why certain proteins (keratins, mucin, ricin, etc.) are digested either with difficulty or not at all.

The Reversibility of Enzyme Action

The definition of a catalyst states that it is a substance which accelerates the speed of a reaction. The catalyst is not believed to initiate reactions, and affects only those reactions that are capable of proceeding at a very slow rate in its absence. Moreover, catalysts do not alter the equilibrium points of reactions. Hence it is logical to assume that catalysts, including enzymes, should be capable of catalysing reactions in both directions and that the direction of the action should be dependent only upon the respective concentrations of substrate and product. The reversible action of ordinary catalysts is well known. With enzymes, demonstration of specific reversibility is frequently a matter of very great difficulty on account of the fact that the equilibrium point is situated near the extreme end of the reaction. Hence the amount of product may be too small to be detected chemically.

In the hydrolysis of sucrose the mutarotation of the two products, glucose and fructose, complicates matters and

may cause a change in the point of equilibrium. The hydrolysis of starch by amylase probably involves the consecutive action of a number of different enzymes upon a number of intermediate dextrins. Here also we should expect to have difficulty in establishing proof of reversibility.

The first accepted proof of the specific reversible action of enzymes was brought forward by Kastle and Loevenhart, who showed that when fresh extracts of the pancreas are treated with a dilute mixture of ethyl alcohol and butyric acid, the odor of ethyl butyrate soon develops, whereas there is no development of the odor of ethyl butyrate if the pancreas extract is first boiled. Since this time a large number of cases of the reversible action of enzymes have been demonstrated. A number of beta glucosides have been synthesized by the action of emulsin, and several alpha glucosides have been prepared by the action of maltase. In dehydrolytic reactions it is necessary to use concentrated solutions in order to limit the amount of water present. The smaller the concentration of water the greater will be the amount of ester formed, as can be foretold from consideration of the equation:

$$\frac{\text{acid} \times \text{alcohol}}{\text{ester} \times \text{water}} = \text{K}$$

In this connection the student should study the application of the mass law to the equilibria of balanced reactions, on page 238.

The reversible action of pepsin has long been suspected from the fact that when concentrated peptone solutions are acted on by pepsin a protein-like precipitate is slowly formed. This is called plastein. Wasteneys and Borsook have recently shown that plastein formation occurs most readily at a pH of 4.00, which is considerably below the optimum acidity for the hydrolytic action of pepsin. Plas-

tein has been shown by chemical analysis to be much more complex than the peptones used for its preparation.

The synthesis of urea from ammonium carbonate has been satisfactorily demonstrated by Kay. Urease was added to a concentrated solution of ammonium carbonate and the material was subjected to continuous extraction by butyl alcohol, boiling under reduced pressure. The material extracted by the butyl alcohol was identified as urea.

It is a curious fact that although plant and animal cells are largely composed of water, synthetic reactions occur in them. Glycogen is deposited in the animal liver and starch is formed in the plant; amino acids are linked together in both plants and animals to form proteins. It is believed that the cell is able to avoid the hydrolytic effect of water upon syntheses by removing the products from the sphere of reaction as soon as they are formed, either by precipitation, or by transference to some other cell.

The Effect of Reaction on Enzyme Action

There exists for each enzyme a certain reaction at which the activity of the enzyme is most pronounced. As the medium is made more acid, or more alkaline, the catalytic power of the enzyme begins to decrease, at first slowly and then rapidly. The addition of still more acid, or alkali, causes complete inactivation, which in many cases is irreversible. The enzymes of the digestive tract are most reactive at approximately the reactions of the secretions in which they occur. This is generally true of enzymes present in blood and in the tissues. However, the autolytic enzymes of the tissues have been found to be favorably influenced by a faintly acid reaction, such as occurs a few hours after death. The table below gives the reaction optima for some of the enzymes whose properties have been most frequently investigated.

enzyme	optinum pH
ptyalin	6.7
pepsin	1.8
trypsin	8.0
erepsin	7.8
amylopsin	6.8
steapsin	8.5
maltase (intestinal)	ca. 7
invertase (intestinal)	5–7
invertase (yeast)	4.5
malt amylase	4.5–5.0
urease	7.0
lipase (castor bean)	ca. 3.

The nature of the substrate is not without influence upon the point of optimum reaction. Casein is more rapidly digested by trypsin at the neutral point than on the alkaline side. The inorganic salts which are present also affect the point of optimum reaction.

It is often useful, and sometimes necessary, to employ buffer salts when estimating the activity of enzyme preparations. Neutral phosphate solution is always added in the determination of urea by urease, or in the estimation of urease activity, the reason being that the ammonium carbonate which is formed soon makes the medium so alkaline that the urease can no longer act.

Kinases

In certain cases enzymes are produced by cells in an inactive state. In this condition they are known as zymogens. Upon secretion they come in contact with some substance that converts them to the active state. Substances having this activating effect are known as kinases. The reaction is not reversible. Pepsin exists in the chief, or central cells, of the gastric mucosa as the zymogen, pep-

sinogen, and is changed into the active pepsin by coming into contact with the hydrochloric acid of the gastric juice. Pepsinogen differs from pepsin in being much less sensitive to the action of alkali. Pancreatic trypsin is present in the pancreas and in the pancreatic juice as the zymogen trypsinogen. The kinase which converts trypsinogen into trypsin is secreted in the intestinal juice and is known as enterokinase. The nature of enterokinase is unknown. The other enzymes of the digestive tract do not exist as zymogens. The reason for the production of pepsin and trypsin as zymogens and their later activation by kinases may be for the purpose of protecting the tissues.

Co-enzymes and Accelerators

A large number of substances have been found to have a specific accelerative, or activating action upon enzymes. The kinases, mentioned above, are distinctive in that they activate in an irreversible manner. The class of activators known as co-enzymes affects enzymes in a reversible manner. In some instances the presence of a co-enzyme is essential for enzyme action, in others the co-enzyme merely increases the activity of the enzyme. Co-enzymes are not destroyed by boiling, and are dialysable. Yeast zymase can be separated from its co-enzyme by filtration through a gelatin filter. The co-enzyme passes through, but the enzyme does not. The enzyme is not active in this case, but becomes so when the filtrate, or when some boiled yeast juice, is added to it. The bile salts, sodium glycocholate and sodium taurocholate, function as co-enzymes for steapsin. However, steapsin is active in the absence of bile salts. In the presence of bile salts the activity of steapsin is increased. The effect of bile salts is possibly due to their solvent action, or to their emulsifying action on the fat. Various inorganic salts have been found to have an accelerative effect upon enzymes. Chlorides are favorable for the action of ptyalin;

phosphates are probably necessary for the action of zymase. In this last case the phosphate is needed for the formation of an intermediate product, hexose diphosphoric ester.

Not all enzymes have been found to require the presence of a co-enzyme for their action, but all enzymes can be accelerated by the addition of some substance or other. Urease requires no co-enzyme, in the strict sense of the word, but its action is aided by buffer salts, by cyanides, proteins, amino acids and by most colloids.

Paralyzers

The ions of heavy metals usually have an inhibiting or destructive action on enzymes. This effect must not be confused with the effect which is due to the acid properties of certain salts. The ions of silver, mercury, and copper are among the most harmful. Urease is extraordinarily sensitive to mercury ions and somewhat so to cupric ions. Lead ions have so little effect upon urease that the enzyme is active even in the presence of a large excess of neutral lead acetate. In some cases anions inhibit enzyme action. The cyanide ion is a powerful poison for catalyase and for invertase. Papain, on the contrary, is activated by cyanide. In the investigation of enzyme action it is often necessary to add a preservative in order to prevent the growth of moulds and bacteria. Some preservatives have a paralyzing action on ezymes and on that account cannot be used. Chloroform inhibits the action of pepsin. Formaldehyde has an inhibiting action on many enzymes. Toluene is the best preservative.

Protectors

Glycerol has a protective action on enzymes and is accordingly used for the preparation of enzyme solutions. Proteins have a protective action. This may be due both to a buffer effect and to a colloidal protective action of proteins. Enzymes are protected also by low temperatures.

Anti-enzymes

After injecting solutions of enzymes into animals antibodies have been found in some cases to appear in the blood. These substances, which are similar to antitoxins, have a specific inactivating action upon the enzyme which caused their formation. Anti-enzymes probably do not directly retard reactions after the manner of anti-catalysts. Anti-emulsin, anti-steapsin and anti-pepsin have been prepared. The intestinal parasite ascaris contains anti-pepsin, anti-rennin and anti-trypsin, which are readily prepared by grinding the worms with sand.

The Preparation of Enzymes

Enzymes can be obtained by grinding plant or animal materials with a meat chopper, or with a mill, and extracting with water, dilute alcohol, or dilute glycerol. The liquid is pressed out and centrifuged, or filtered. When dealing with yeast cells or bacteria the material is ground with quartz sand, or infusorial earth, in order to rupture the cell walls. The ground mass is placed in a cloth bag and subjected to great pressure in a Buchner press. Rupture of the cells can be brought about by drying the material (yeast) at 40° C. and then heating to 100° C. Washing the material with acetone or with absolute alcohol is also effective. Cellular material can be allowed to autolyse in the presence of an antiseptic. Autolysis causes rupture of the cell walls. The enzyme can be then extracted and filtered.

The best solvent for enzymes is water, but dilute alcohol is usually to be preferred as it extracts less of the impurities and the alcoholic extracts are very much easier to filter than are aqueous extracts. When enzymes are present in one of the digestive fluids such saliva, gastric juice, or pancreatic juice, it is best to obtain them from this source.

Pure gastric juice can be obtained from an isolated fundic sac, according to the method of Pawlow. Pancreatic juice can be obtained by inserting a cannula directly into the pancreatic duct. The pancreas can be made to secrete by injecting secretin into the blood stream.

Pepsin is usually obtained by extracting the hashed mucous coat of the stomach with dilute hydrochloric acid. Trypsin, amylopsin and steapsin are prepared by hashing fresh pancreas and extracting with water or with dilute alcohol, for several days at a low temperature. Autolysis liberates the enzymes. Erepsin, invertase, maltase and lactase can be obtained by grinding the epithelial coat of the duodenum with sand, extracting with water and filtering. Yeast invertase is prepared by allowing pressed yeast to autolyse for several days in contact with chloroform. The chloroform aids the process of autolysis. The product is treated with lead acetate and filtered. The filtrate is treated with hydrogen sulphide, filtered and dialysed.

The Purification of Enzymes.—The purification of enzymes is an extremely difficult task. The amount of enzyme present is presumably very small. The enzyme is of colloidal nature, which makes purification difficult on account of the tendency of colloids to adsorb substances. The unstable nature of enzymes precludes the use of many reagents and methods of purification. Moreover, as the process of purification proceeds the enzyme becomes more and more unstable.

Adsorption methods have been largely used for the purification of enzymes, but have been only partially successful. By adsorption with Lloyd's reagent, kaolin, aluminum hydroxide, calicum phosphate, etc., it is possible to remove either the enzyme, or some of the impurities, depending on the nature of the enzyme and the reaction of the medium. For example, urease is well adsorbed at a pH of 5.8. After centrifuging off the adsorption complex and washing it, the urease can be liberated by adding neutral phosphate

solution. Since urease is not readily adsorbed from neutral solution, it is possible to remove some of the impurities by shaking neutral urease solutions with adsorbents. Poor yields are obtained by adsorption methods, as it is not always possible to recover all of the enzyme from the adsorbent, and it becomes increasingly difficult to do so as the process of purification proceeds. McMeekin and Koch have purified pepsin by adsorbing it from acid solution upon coagulated egg white. The pepsin was then liberated by extracting with a faintly acid buffer solution. The adsorption of some enzymes is not influenced greatly by the reaction of the medium. This is true for zymase.

Enzymes can be precipitated by alcohol, or can be salted out of solution by the addition of ammonium sulphate, or sodium sulphate at 30° C. Such methods effect a moderate amount of purification in some cases but more often are detrimental. Enzymes can be partly purified by dialysing. Dialysis removes diffusible substances and such cations and anions as are not chemically bound to the enzyme, or to proteins that are present.

Urease can be chemically precipitated both by lead acetate and by tannic acid without inactivation. The lead urease can be washed with sodium sulphate without decomposition. Upon the addition of oxalate, or neutral phosphate solution the urease is liberated. The urease tannate cannot be decomposed. Urease can be almost completely precipitated by cooling its 35 per cent alcoholic solution to −10° C. and centrifuging, provided the reaction is about pH 5.8. The precipitate is stirred up with a little dilute neutral phosphate solution and the globulins which are present are allowed to crystallize out in the ice chest. After centrifuging, the urease remaining in solution is rendered insoluble by standing in 30 per cent alcohol. The insoluble urease is probably either a denatured protein or a mixture of insoluble, inactive urease combined with active urease.

As this book goes to the publisher I am able to announce that I have recently isolated urease in crystalline form. The crystals are octahedral and therefore belong to the isometric system. They dissolve readily in water and give all of the protein tests. The percentage of nitrogen is not far from 17 and the enzyme activity is a little more than 100,000 units per gram of dry material. In other words,

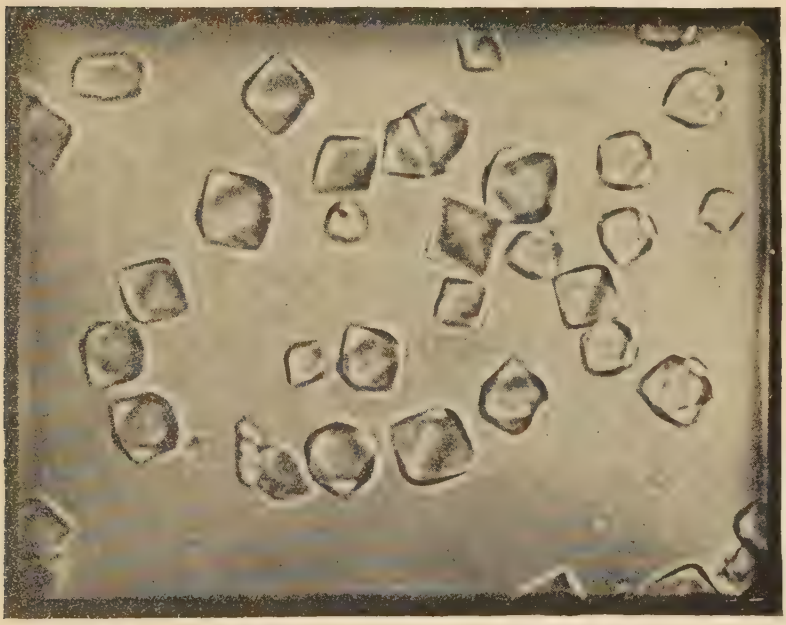


FIG. 4. Crystals of the enzyme urease (magnified 1420 diameters).

1 gram of the crystals will produce from a solution of urea, buffered by phosphates, 100,000 mg. of ammonia nitrogen in 5 minutes at 20° C. The material is probably a globulin, for although it is soluble in distilled water a precipitate is formed on the addition of carbonic acid and this dissolves when neutral phosphate solution is added. The octahedral crystals have been recrystallized and have been shown to retain their activity, which, within the limits of experimental error, remains 100,000 units per gram.

Chapter XI

THE PHYSICAL CHEMISTRY OF PROTOPLASM

Introduction

A familiarity with certain phases of physical chemistry and with certain physico-chemical methods is a *sine qua non* for the intelligent study of a large number of biological problems. The mere knowledge of the constituents of plants and animals and the chemical reactions that occur in the cells is not by itself a sufficient explanation of biological phenomena. The application of physical chemistry to biology during recent years has resulted in the creation of new conceptions and explanations of plant and animal processes. The student requires an understanding of some of the fundamentals of physical chemistry if he is to grasp these applications to biology.

Since protoplasm is partly composed of colloidal gels and sols, one must be familiar with different states of matter and with the general properties of matter in the colloidal state. In the study of kidney function and the secretion of urine the meaning of the term "osmotic pressure" becomes significant. The literature on the buffers of the blood, the transportation of carbon dioxide and the regulation of neutrality cannot be understood unless one is familiar with the mass law, the theory of ionization and the dissociation of acids and bases; while work with proteins brings one face to face with such terms as "adsorption," "iso-electric point," "dispersion" and "hydrophil colloid."

Osmotic Pressure

Diffusion

If a heavy gas, like chlorine, is placed in the bottom of a bottle containing air, it will diffuse upward against gravity. At the same time the air will diffuse downward into the chlorine. Ultimately the two gases will become uniformly mixed in every part of the bottle. This diffusion is brought about by the difference in partial pressures; at the start there is no air pressure in the chlorine and no chlorine pressure in the air.

Osmosis

If a solution of copper sulphate is placed in a beaker and overlaid with distilled water, the copper sulphate will diffuse slowly upward, while the water will diffuse downward, until the concentration of copper sulphate is uniform throughout the liquid. In this instance the diffusive force is called "osmotic pressure" and is due to the attractive force (or cohesion) between the dissolved substance and the solution. Osmotic pressure can be readily demonstrated by placing a parchment bag containing a concentrated solution of some substance in a beaker of distilled water. Since the viscosity of water will be less than the viscosity of the solution, water will diffuse into the solution more rapidly than the solute diffuses into the water. Hence the level of solution will rise. Ultimately gravity will cause this level to descend to that of the outer liquid.

Measurement

Pfeffer's Method.—In 1877 the botanist Pfeffer showed how osmotic pressure could be measured. Pfeffer had observed that concentrated solutions caused the protoplasm of certain vegetable cells to shrink (plasmolysis), while distilled water caused the protoplasm to swell and burst the

cellulose walls (plasmoptysis). Pfeffer's apparatus consisted of a battery pot of unglazed porcelain tightly fitted with a closed mercury manometer. The pores of the pot were filled with a precipitate of copper ferrocyanide. This was accomplished by allowing copper sulphate to diffuse into the wall of the cell from the inside and potassium ferrocyanide to diffuse in from the outside. Copper ferrocyanide is permeable to water but is practically impermeable to many dissolved substances. It can therefore be classed as a semipermeable membrane. When the apparatus (osmometer) was filled with the solution to be tested and placed in distilled water, the attraction between the water and the dissolved substance caused the water to diffuse into the cell until the attractive force was balanced by the pressure of the column of mercury in the manometer.

Pfeffer's results were sufficiently accurate to show that osmotic pressure is proportional to the concentration of the dissolved substance and to the absolute temperature. From consideration of these results Van't Hoff soon afterwards established the following theory of solutions:

A dissolved substance behaves like a gas occupying the same volume and obeys analogous laws.

The gas laws are three in number:

1. Boyle's law states that at a constant temperature the pressure is inversely proportional to the volume (or directly proportional to the concentration).

2. Gay-Lussac's law states that with constant pressure the volume will increase $\frac{1}{273}$ for every degree rise in temperature above 0° C.

3. Avogadro's hypothesis states that under the same condition of temperature and pressure gram molecular weights of all gases occupy the same volume. (At 0° C. and 760 m.m. pressure, this volume is 22.4 liters and is consequently known as the gas constant, or R.)

According to Van't Hoff the osmotic pressure of a solution is analogous to the pressure of a gas. Hence the osmotic pressure of any solution will be inversely proportional to its volume and directly proportional to its concentration; the osmotic pressure will increase $\frac{1}{273}$ for each rise in temperature of 1 degree above 0° C. Finally, all solutions containing the same number of dissolved particles will, at any given temperature, have the same osmotic pressure. Since a gram molecule of any gas occupying 22.4 liters will at 0° C. exert a pressure of 760 m.m. of mercury, a gram molecule of dissolved substance occupying 22.4 liters will at 0° C. have an osmotic pressure of 760 m.m. of mercury, or 1 atmosphere. Conversely a gram molecule of dissolved substance occupying a volume of 1 liter will have an osmotic pressure of 22.4 atmospheres at 0° C.

The direct method of determining osmotic pressure, although improved by the apparatus of Morse and Frazer, and of Berkeley, is nevertheless a difficult procedure. Osmotic pressure can be more easily measured by the cryoscopic method.

Cryoscopic Method.—This method depends upon the fact that the freezing point of the solvent is depressed in direct proportion to the number of dissolved particles. It has been found that an aqueous solution, containing 1 mol of dissolved substance per liter, has a freezing point of −1.85° C. The freezing point of benzene is depressed 5.0° by the presence of 1 mol of dissolved substance; the freezing point of acetic acid is depressed 3.9°. Since the freezing point depression is dependent upon the number of dissolved particles and not upon the kind of particle, a determination of the freezing point of a solution of known concentration can be used to calculate the molecular weight of the substance dissolved (provided that the substance is neither ionized nor associated).

Ebullioscopic Method.—Dissolved substances elevate the boiling points of the solvent, provided that the dissolved substance is not volatile. For water the gram molecular elevation is 0.52°, for ethyl alcohol 1.15°, for chloroform 3.9°. Determination of the boiling point is somewhat more difficult than determination of the freezing point on account of the phenomenon of superheating. Moreover, the method cannot be used with solutions containing protein, bicarbonates or other substances that will be decomposed by heat.

The Mass Law

The mass law can be used to define the position of equilibrium of a balanced reaction (a balanced reaction is one that does not go to completion, see p. 216). A well-known example is the esterification of acetic acid by ethyl alcohol:

$$CH_3COOH + HOC_2H_5 \rightleftarrows CH_3COOC_2H_5 + H_2O$$

If one mixes 1 mol of glacial acetic acid with 1 mol of absolute ethyl alcohol the two compounds will react until two-thirds of a mol of ethyl acetate and two-thirds of a mol of water have been formed. The reaction then ceases and is said to have reached its point of equilibrium. The same point of equilibrium can also be attained if one mixes one mol of ethyl acetate with one mol of water.

Equilibrium Constant.—With all balanced reactions the mass law states that the product of the concentrations (expressed in mols per liter) of the substances on one side divided by the product of the concentrations of substances on the other side is equal to a constant. Expressed as an equation:

$$\frac{[\text{acid}] \times [\text{alcohol}]}{[\text{ester}] \times [\text{water}]} = K$$

The acid and alcohol will react together with a velocity, which we will call V_1. This velocity is proportional to the

concentration of acid and alcohol; or it is equal to the product of the concentrations of acid and alcohol, multiplied by some constant. This constant we will call K_1. So that:

$$[\text{acid}] \times [\text{alcohol}] \times K_1 = V_1$$

Starting with ester and water we can see that their velocity of reaction, which we will call V_2, is equal to the product of their concentrations, multiplied by another constant, K_2, or:

$$[\text{ester}] \times [\text{water}] \times K_2 = V_2$$

At the point of equilibrium the velocity in one direction is equal to the velocity in the other direction, or $V_1 = V_2$, or:

$$\frac{[\text{acid}] \times [\text{alcohol}]}{[\text{ester}] \times [\text{water}]} = \frac{K_2}{K_1} = K$$

One constant divided by another constant gives a constant, which can be called K. This is the equilibrium constant. It represents $\frac{V_1}{V_2}$, the ratio of the velocity constants in the two opposite directions. The value of this velocity constant has been found experimentally to be 0.25 for this particular reaction.

Since the amounts of acid and alcohol mixed together at the start do not have any influence upon the constant it is possible to calculate the concentrations of the products of the reaction if the concentrations of the reacting substances and the value of the constant are known. Starting with 1 mol of acetic acid and 7 mols of ethyl alcohol we would have

$$\frac{(1-P)\ (7-P)}{P^2} = 0.25,$$

where P is equal to the mols of water or of ester formed. By solving the quadratic equation, $0.75\ P^2 - 8\ P + 7 = 0$, we find that P = 96 per cent of a mol

The mass law can be used in an analogous manner to calculate the degree of dissociation of water, acids, or bases (see pages 245 and 246), provided the equilibrium constant, or as it is called in this instance, the dissociation constant, is known.

Electrolytic Dissociation

Acids, bases and salts differ from other compounds (alcohols, ketones, aldehydes, etc.) in the following respects:

1. They depress the freezing point and elevate the boiling point of their solutions more than the calculated amount.
2. They exert a greater osmotic pressure than would be expected.
3. Their solutions are conductors of electricity.

Isotonic Coefficient.—According to the theory of Arrhenius, acids, bases and salts dissociate in water into cations and anions:

$$NaCl \rightleftarrows \overset{+}{Na} + \overset{-}{Cl}$$

If the reaction goes to completion, the solution will contain twice as many particles as there would be if no dissociation had taken place. A molar solution of sodium chloride depresses the freezing point 3.35° instead of the expected 1.85°. The solution acts as if it contained $\frac{3.35}{1.85}$, or 1.8 mols per liter. This value is called the isotonic coefficient. The isotonic coefficient increases with dilution. Thus, the isotonic coefficient for 1 per cent sodium chloride is 1.9, for 0.27 per cent sodium chloride it is practically 2.0.

The isotonic coefficient can be used to calculate the degree of dissociation, using the following equation:

$$X = \frac{i-1}{n-1}$$

where i = isotonic coefficient
x = degree of dissociation
n = number of parts into which the salt dissociates

Applying the equation to molar sodium chloride we have:

$$X = \frac{1.8-1}{2-1},$$

or 0.8, which is 80 per cent of 1 mol.

Conductivity Measurements

Conductivity is the reciprocal of resistance, or $\frac{1}{R}$, where R stands for resistance. The electrical conductivity of a solution depends upon the number of ions present and is not influenced by the undissociated molecules. Conductivity increases relatively with dilution because the degree of ionization is increased by dilution. At infinite dilution an ionizable substance can be considered to be completely dissociated. Accordingly the degree of ionization can be found by dividing the conductivity of a mol of solute at the given dilution by its conductivity at infinite dilution. The conductivity of a mol (or gram equivalent) is known as the equivalent conductance. The value for conductance at infinite dilution can be found by determining values for known dilutions and then extrapolating. Another method is to add together the conductance values for the individual ions. For an explanation of the latter method the student is referred to Le Blanc's "Text Book of Electro-Chemistry," or to any text book of physical chemistry. The equivalent conductance of a solution can be obtained by finding the resistance of a centimeter cube. The reciprocal of this value is the specific conductance. To obtain the equivalent conductance it is necessary to multiply this value by the number of cubic centimeters of the solution that would contain one mol of the dissolved substance.

A more practical method of determining equivalent conductance is as follows:

A cell containing platinum electrodes of any size or shape is used. The cell is filled with solution A, the equivalent

conductance of which is already known, and its resistance is measured. The resistance of solution B, of which the equivalent conductance is unknown, is then measured in the same cell. The equivalent conductance can be calculated, using the equation given below:

$$\frac{\text{resistance of A}}{\text{resistance of B}} = \frac{\text{equivalent conductance of A}}{\text{equivalent conductance of B}}$$

Surface Tension

Surface tension is the unbalanced force of cohesion, or molecular attraction, which exists at a surface. Within a liquid (or a solid) the attraction of molecules for one another exerts a pull in all directions, while at the surface the pull outwards is lacking. Consequently this lack of balance causes a liquid to act as if it possessed a stretched elastic film. Rain drops, soap bubbles, air bubbles and drops of mercury assume a spherical shape on account of the existence of surface tension, i.e., on account of the inward pull of cohesion from all exterior directions. The capillarity or rise of water and the depression of mercury in tubes, notably those of capillary bore, are caused by surface tension.

Surface tension decreases with rise in temperature and ceases to exist at the boiling point. When a liquid is heated in a sealed tube to its critical point the surface suddenly disappears and surface tension no longer exists. Surface tension increases with decrease in temperature. When a liquid solidifies the surface tension becomes very great; just how great it is difficult to say, as there is no method for measuring the surface tension of solids.

Of the many methods for measuring surface tension only the simplest will be described here. This method consists in counting the number of drops of the liquid delivered by a stalagmometer. The stalagmometer is then filled with

distilled water and the number of drops of water delivered are counted. The equation is:

$$\text{relative surface tension} = \frac{\text{specific gravity of solution} \times \text{number of drops of water}}{\text{number of drops of solution}}$$

As the result is only relative it must be multiplied by the surface tension of water. This is 73 dynes at 18° C. Below are given some surface tension values in dynes per centimeter:

substance	temperature	surface tension
mercury	15°C	436
water	18°	73
glycerol	20°	65
ethyl alcohol	18°	22
ether	20°	16.5

The hydrophil colloids (proteins, polysaccharides, soaps, phosphatides, etc.) greatly lower the surface tension of water and, as Gibbs has pointed out, become concentrated in the surface. The class of substances referred to have a lower cohesion than water. Substances like salts have a very high cohesion and their effect is to raise the surface tension. Salts are less concentrated in the surface of a liquid than in the interior, since they would have to do work to move into the surface film.

The presence of small amounts of hydrophil colloids makes water foam readily when it is shaken, or stirred, since much less energy need be expended in increasing the surface area. The increased viscosity of the solution also aids in the formation of foam.

Likewise the emulsification of an oil is aided by the presence of a substance which lowers the surface tension, which

increases the viscosity and which protects the emulsified particles by forming a film about them.

The effect of surface tension makes difficult the formation of bubbles of steam when water boils, if no air bubbles are present to serve as a starting point. A glass tube, in which water has been repeatedly boiled, contains no air in its pores and this causes bumping (Folin). This trouble can be avoided by heating the tube in a flame, to drive the water out of the pores. After several hours the pores will be found to contain a fresh supply of air.

The Hay test for bile in urine depends upon the decrease in the surface tension of the urine caused by the presence of the bile acids. Here we find that finely powdered sulphur is not supported by the surface of the liquid if bile is present.

The Hydrogen Ion Concentration

Acid, Alkali and Neutral.—An acid is a substance that is capable of furnishing hydrogen ions when it is dissolved in water or in some other suitable solvent. Strong acids like hydrochloric, nitric and sulphuric furnish large quantities of hydrogen ions, while weak acids like acetic and carbonic ionize only to a slight degree. Alkalis are characterized by their ability to furnish hydroxyl ions; solutions of the strong alkalis (sodium hydroxide) contain large quantities of hydroxyl ions, while solutions of weak alkalis (ammonium hydroxide) contain only small numbers of hydroxyl ions. A neutral solution is one which contains an equal number of hydrogen and hydroxyl ions.

Relation of $\overset{+}{H}$ and $\overset{-}{OH}$.—The relation of hydrogen to hydroxyl ions in any solution is a reciprocal one; as the concentration of hydrogen ions increases, the concentration of hydroxyl ions decreases and vice versa. This reciprocal relationship of hydrogen and hydroxyl ions is due to the

reaction for the ionization of water, which is:

$$H_2O \rightleftarrows \overset{+}{H} + \overset{-}{OH}$$

or expressed as an equation:

$$\frac{[\overset{+}{H}] \times [\overset{-}{OH}]}{H_2O} = K$$

where K is the dissociation constant of water. Measurements of the conductivity of pure water at 22° C. give K a value of 1×10^{-14}. From this value we can calculate that both $\overset{+}{H}$ and $\overset{-}{OH}$ have a value of 1×10^{-7}. In other words, one liter of pure water at 22° C. will contain 1×10^{-7} gram molecules of hydrogen ions and the same quantity of hydroxyl ions, or, expressed in grams, 1×10^{-7} grams of hydrogen ions and 17×10^{-7} grams of hydroxyl ions.

A normal solution of hydrochloric acid contains 35.46 gm. of chlorine and 1.008 gm. of hydrogen per liter. If all of the hydrogen were ionized, the concentration of hydrogen ions per liter would be 1 mol. This is not true, as normal hydrochloric acid is only 90 per cent ionized. Tenth normal hydrochloric acid is more completely ionized, and the hydrogen ion concentration is not far from 0.1 mol, or 1×10^{-1} per liter. With hundredth normal hydrochloric acid the hydrogen ion concentration is practically 0.01 or 1×10^{-2}, with thousandth normal it is practically 0.001 or 1×10^{-3}, and so on.

Since the relationship of the hydrogen ion to the hydroxyl ion is reciprocal, it can be seen that if normal sodium hydroxide were completely ionized, its hydrogen ion concentration would be 1×10^{-14}. If tenth normal sodium hydroxide were completely ionized the hydrogen ion concentration would be 1×10^{-13}. Hundredth normal sodium hydroxide, which is almost completely ionized, has a hydro-

gen ion concentration of 1×10^{-12}. Pure water, with a hydrogen ion concentration of 1×10^{-7}, is ten millionth normal acid and is also ten millionth normal alkali. As the temperature of water increases, its dissociation constant increases in value, and the concentration of both hydrogen and hydroxyl ions increases. As water is both an acid and a base at very high temperatures, it is easy to see why substances should be hydrolysed when subjected to the action of superheated steam.

Notation.—The hydrogen ion concentration, or cH, involves the use of many figures when values are written as decimals. For this reason it is usually expressed as powers of 10. A still greater saving of space is accomplished if only the power is written, omitting the minus sign. The value now becomes not cH but pH, meaning power of the hydrogen ion. Thus the pH of pure water is 7, the pH of thousandth normal hydrochloric acid is 3 and the pH of millionth normal sodium hydroxide is 8. Since these values are powers, they are also logarithms (to the base 10). In changing a hydrogen ion concentration of 1.8×10^{-7} into pH it is only necessary to look up the logarithm of 1.8 and to subtract this value from 7.

Dissociation Constants.—While the hydrogen ion concentration of strong acids in dilute solution can be deduced directly from the per cent of acid present, this is not true of weak acids, which undergo only partial ionization. With weak acids it is possible to calculate the hydrogen ion concentration from the dissociation constant of the acid in question. The dissociation constant of acetic acid is 1.8×10^{-5}. Using the equation:

$$\frac{[\overset{+}{H}] \times [CH_3\overset{-}{C}OO]}{[CH_3COOH]} = K$$

where K is the dissociation constant and $[CH_3COOH]$

signifies the mols of acetic acid per liter, we have for tenth normal acetic acid:

$$\frac{x^2}{0.1} = 1.8 \times 10^{-5}$$

which gives us a pH of 2.89.

When a weak acid exists in solution together with its own salt the ionization of the acid is greatly depressed. In this instance we can use the approximate formula:

$$[\overset{+}{H}] = K \frac{\text{mols of acid}}{\text{mols of salt}}$$

This formula can be applied to dilute solutions where the salt is very largely ionized, but for concentrated solutions the value for mols of salt must be multiplied by the degree of dissociation. Let us take as an example a solution containing 1 mol of sodium acetate, which is 53 per cent dissociated at this concentration, and 0.1 mol of acetic acid. The equation will be:

$$[\overset{+}{H}] = 1.8 \times 10^{-5} \frac{0.1}{1(0.53)},$$

which gives a cH of 3.4×10^{-6}, or a pH of 5.47.

The Term pK_1.—Instead of the equation mentioned above one may substitute for

$$\frac{K}{\text{degree of dissociation}}$$

the value K_1. The equation may then be written:

$$\overset{+}{H} = K_1 \frac{\text{mols of acid}}{\text{mols of salt}}.$$

Since pH is equivalent to the minus logarithm of the hydrogen ion concentration we may say:

$$pH = -\log K_1 - \log \frac{\text{mols of acid}}{\text{mols of salt}},$$

or

$$pH = pK_1 + \log \frac{\text{mols of salt}}{\text{mols of acid}}.$$

When the concentrations of salt and acid are equal, pH= pK_1. For phosphates this value is 6.8; for carbonic acid and bicarbonate it is 6.1. The term pK_1 was introduced by Hasselbalch and is of importance in connection with the study of buffers. It is the point of maximum efficiency of a buffer solution.

Measurement of cH

It is possible to measure the hydrogen ion concentration of any solution either electrometrically, or colorimetrically. The electrometric method is the more difficult, while the simpler colorimetric method is subject to errors caused by the effect of proteins and salts upon the indicators.

The Electrometric Method.—When a metal is placed in water there is a tendency for the atoms of the metal to ionize. This tendency is called the electrolytic solution tension. If ions of the metal are present in solution they have a tendency to separate out on the metal. This is due to the osmotic pressure. When copper is placed in distilled water, the tendency to ionize is strong and the metal is left with a negative charge. If copper is placed in a concentrated solution of copper sulphate, the osmotic pressure overcomes the electrolytic solution tension and the copper will acquire a positive charge. We can picture a system like the following:

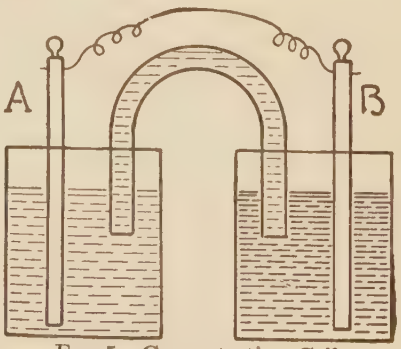


Fig. 5. Concentration Cell.

Where a copper electrode in a dilute solution is connected by a U tube, full of liquid, to a copper electrode in a concentrated solution of copper sulphate, this is known as a "concentration cell." If electrodes A and B are connected with a wire, so that the electric current is allowed to pass, copper ions will be given off from electrode A and copper ions will deposit upon electrode B until the concentration of copper sulphate in both solutions is equal. The sulphate ions will migrate from the concentrated to the dilute solution.

It can easily be seen that the electromotive force of such a cell will be proportional to the difference in the concentration of copper sulphate in the two cells and that it will decrease in value as the difference in concentration diminishes.

If we can imagine two electrodes composed of hydrogen, one electrode immersed in a dilute solution of acid and the other in a stronger solution of acid, we can see that in this case an electric current will be produced, the voltage depending upon the difference in concentration. When gaseous hydrogen is allowed to come in contract with an electrode of gold which has been coated with platinum black (very finely divided platinum deposited by electrolysis from a solution of chlorplatinic acid), the electrode acts as if it were made of metallic hydrogen, and is called a hydrogen electrode. It is possible to construct a system composed of two cells, one containing the solution of unknown hydrogen ion concentration, the other containing 1.25 normal hydrochloric acid, which has a hydrogen ion concentration of one mol per liter. Hydrogen electrodes are placed in both cells. The cells are connected by siphons through a beaker containing potassium chloride. This salt is chosen because the potassium ion moves in an electric field at nearly the same velocity as the chlorine ion and for this reason there is caused no difference in potential at the

boundary of the potassium chloride solution (contact potential). The electromotive force of the cell can be measured by balancing the current against that of a cell of known voltage, adding resistance coils until no current flows. This point can be detected by means of a capillary electrometer. In practice it has been found much simpler to use a calomel electrode instead of a cell containing normal hydrochloric acid.

Inasmuch as an electric difference in potential can be measured to within one millionth of a volt, the determination of the hydrogen ion concentration is capable of a high degree of accuracy. For a description of the apparatus and of the method of procedure the student is referred to Findlay, "Physical Chemistry for Students of Medicine."

The Indicator (Colorimetric) Method

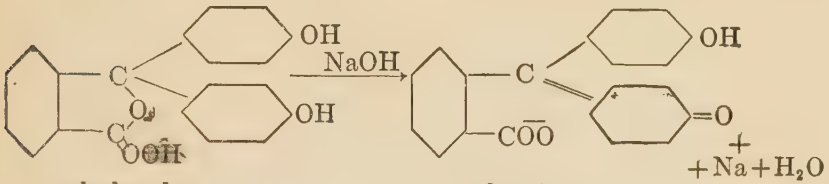
This method depends upon the fact that certain colored, organic compounds, known as indicators, are so influenced by the hydrogen ion concentration that their color varies with changes of pH. Indicators are always either acids or bases and are capable of forming salts. The mass law applies to indicators as well as to other acids and bases and the proportion of free indicator and its salt with an acid or a base can be expressed by the equation:

$$pH = pK + \log \frac{[\text{salt}]}{[\text{acid, or base}]}$$

This means simply that the color of the indicator will depend upon the pH of the solution in which it is dissolved.

The change in color of indicators was formerly believed to be due to the process of ionization but it is now thought that ionization is only incidental to the change in color and that the change is caused by a tautomeric rearrangement of the indicator molecule that is made possible through ionization. Let us take the indicator phenolphthalein as an example. Phenolphthalein is a weak acid and as such is

colorless. Upon the addition of alkali the salt is formed and this is colored intensely red even in the dry state. The reaction is:



colorless lactone colored anion

The color of the anion of the salt of phenolphthalein is due to the quinoid structure. Other indicators exist that possess one color in acid solution and another color in alkaline solution. In cases of this sort the change of color is usually brought about by a tautomeric rearrangement involving the production of a quinoid linkage. The quinoid form is usually more intensely colored than the other form.

Individual indicators change color at different hydrogen ion concentrations depending upon their intrinsic acid or basic nature and it is therefore possible to choose an indicator to cover any range of acidity or alkalinity. According to Sörenson tropæolin has a range from the pH of 1.4 to 2.6, methyl orange from 3.1 to 4.4, methyl red from 4.2 to 6.3, neutral red from 6.8 to 8.0, phenolphthalein from 8.3 to 10.0 and thymolphthalein from 9.3 to 10.5. Phenol red has a range from pH 6.8 to 8.0 and has been much used for the determination of the hydrogen ion concentration about the neutral point. Some indicators change color over a short range of the pH scale, while others change color gradually. The former are to be preferred for titration while the latter are useful for the determination of the pH because of their wide range.

For the determination of hydrogen ion concentration by the indicator method a series of buffer solutions is made up, covering the desired range. The pH of these solutions can be calculated from the dissociation constants of the

acids or bases used, or can be actually checked up by the electrometric method, but in most cases if pure reagents are used it is safe to rely on figures given in text books. Buffer solutions are used because they oppose changes in pH that may be caused by traces of impurities, such as carbonic acid absorbed from the air, or alkali given off by glass. Buffers are mixtures of weak acids and their salts or of weak bases and their salts. Some of the buffers most frequently used are mixtures of primary and secondary phosphates, of sodium acetate and acetic acid and of acid potassium phthalate and sodium hydroxide. The acid component and the alkaline component are mixed in different proportions to give a series of pH values. These are placed in tubes of pyrex glass of uniform bore and exactly the same volume of neutral indicator solution is added to each tube. The tubes are then closed with paraffined stoppers. The unknown solution is pipetted into a tube of the same bore as that of the standards, using the same volume that was used in making up the standards. The same amount of indicator is now added and after mixing the comparison is made. If the color of the unknown falls within the color given by two of the standard tubes the pH can be interpolated.

The indicator method is convenient and rapid and in most cases is sufficiently accurate. However, errors are caused by the presence in the unknown solution of salts, proteins, precipitates, or colored substances. The standards must be made rather frequently as they soon deteriorate by fading, by the growth of micro-organisms, or by other changes.

Colloids

The Colloidal State

Animal and vegetable cells are composed largely of colloidal, or glue-like substances. These are partly liquids, or

sols, and partly jellies, or gels. The polysaccharides, the phosphatides and the proteins form colloidal solutions with large amounts of water, and form gels when sufficiently concentrated.

It was Thomas Graham who first noted the distinction between the colloidal state and the crystalloidal state. He noted that colloidal material did not diffuse readily, did not crystallize, and often formed a jelly.

It must be stated that while certain classes of substances, like the proteins, or the higher carbohydrates, always form colloidal solutions, the word colloidal must be considered as defining a physical state. Any substance can exist in a colloidal state. Sodium chloride can be prepared as a colloidal solution in benzene. When anhydrous sodium carbonate is stirred with glacial acetic acid it forms a stiff gel of sodium acetate.

It is possible to differentiate colloidal states, depending upon whether the dispersed phase and the dispersing phase happen to be solids, liquids, or gases. In the case of smoke we have a solid suspended in a gas. In fog the dispersed phase is a liquid. Again, ruby glass represents a solid (metallic particles of gold) in a solid (glass). It is not possible to have a colloidal dispersion of a gas in a gas.

A colloidal system differs from a crystalloidal system because it is heterogeneous and not homogeneous. We can think of a colloidal system as being composed of particles of very great molecular weight (proteins), or of aggregations of a great many particles of low molecular weight (colloidal gold) suspended (dispersed) in some medium. A true solution, such as glucose in water, is practically homogeneous.

There is no sharp division between the colloidal and the crystalloidal state. The lower dextrins are intermediate; they diffuse slowly, and are much more difficult to precipitate than such a substance as starch.

Suspensoids and Emulsoids

Colloidal solutions are conveniently divided into two groups: suspensoids and emulsoids. The suspensoids or suspension colloids are suspended in a medium, and the suspended particles are kept from settling out by an electric charge, either positive or negative. A suspension colloid has very little attraction for the medium in which it is suspended (the dispersion means) and does not greatly affect such physical properties of the solution as viscosity, or surface tension. Suspension colloids are, in other words, "hydrophobic." Examples of colloidal suspensions are: colloidal gold, colloidal arsenious sulphide and colloidal gum mastic.

Emulsion colloids have a very considerable effect upon the physical properties of the solution, and are known as "hydrophilic"; small amounts increase the viscosity and lower the surface tension. The emulsion colloid has a considerable attraction for the solvent (dispersion means), and is not so readily precipitated as the suspension colloid. The particles of an emulsion colloid usually bear an electric charge, but neutralization of this charge does not necessarily cause the precipitation of the particles.

Emulsion colloids and suspension colloids alike have very little effect upon the osmotic pressure, the boiling point, the freezing point and the electrical conductivity of the solution in which they exist. This is due to the fact that the number of particles is relatively small in comparison to the number of particles (molecules or ions) in a crystalloidal solution.

Some examples of emulsion colloids are gelatin, albumin, glycogen, and soap solutions.

It is possible to convert an emulsion colloid into a suspension colloid by changing the dispersion medium. Thus a water solution of agar-agar when poured into alcohol, is converted from the emulsoid to the suspensoid state.

When soap solution is poured into a sufficient amount of alcohol, it is changed from an emulsion colloid to a crystalloid.

Identification of Colloids

The best tests of the colloidal state are:

1. The inability of the material to diffuse through a parchment membrance,
2. The Tyndall phenomenon.

The Tyndall Phenomenon.—The Tyndall phenomenon is the diffuse dispersion of light by colloidal particles. If a colloidal suspension is held between the eye and a source of light, it appears clear, provided that it is not too concentrated. When a colloidal suspension is held away from the source of light over a dark background, it is seen to be cloudy, or opalescent, provided the particles are not too small, or too few in number. The Tyndall phenomenon is observed when a beam of sunlight enters a darkened room. In this instance it is the particles of dust in the atmosphere that make the path of the ray visible. In a perfectly dust-free atmosphere no ray of light can be seen.

The Ultramicroscope.—The ultramicroscope of Zsigmondy utilizes the Tyndall phenomenon and makes it possible for the observer to detect individual colloidal particles by reflected light. The particle itself is not seen but only the reflected light given off by the particle. We see the stars in exactly the same manner.

The ultramicroscope consists of a high-power microscope fitted with an oil immersion objective, bearing upon a small glass chamber containing the colloidal solution. An intense beam of light is focused upon this colloidal solution, passing in a horizontal line, so that no light, except that which is reflected from the particles, reaches the observer's eye.

The Dark-Field Microscope.—Except for the minutest particles, dark-field effects like those of the ultramicroscope

may be obtained more easily with an ordinary microscope supplied with a special reflecting condenser, which as shown in the figure, gives a brilliant light at so great an angle that none of it enters the objective directly. The objects are mounted on an ordinary microscopic slip of the correct thickness and a cover-glass, employed as usual. Immersion oil must be put on the upper face of the condenser to give immersion contact between the lower face of the glass slip and the condenser, and if an oil immersion objective is used, there must be immersion liquid between the lower end of the objective and the cover-glass. With most dark-field condensers the objective used must not have an aperture above 0.80 to 0.85. Special oil immersion objectives are made with the correct aperture. Ordinary oil immersion objectives may be used by inserting a reducing diaphragm above the back combination of the objective.

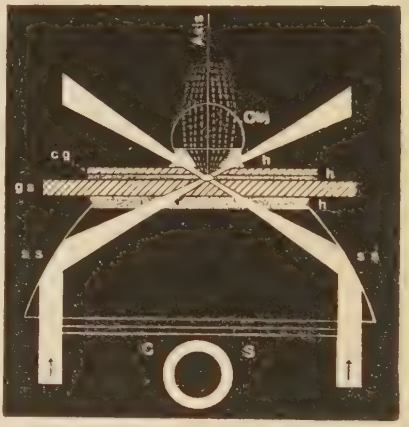


FIG. 6.—Diagram of a Paraboloid, Dark-field Condenser to Show the Course of the Light Cone, and the Light Deflected from the Object into the Objective.

C S Central stop to block the part of the light of aperture below *1.00 NA*.

S S, S S Silvered parabolic surface.

h, h, h Homogeneous liquid between condenser and slip, Canada balsam enclosing the object, and the homogeneous liquid between the objective and cover-glass. (The object may be mounted in air or any liquid.)

g s Glass slip on which the object is mounted; it must be of a thickness to bring the focus of the cone of light on the object.

c g Cover-glass.

Obj Front lens of the objective.

[*From* Gage: The Microscope.]

Microns, Ultramicrons, Amicrons.—Colloidal particles can be classified according to size and visibility. Microns are particles large enough to be visible in the ordinary microscope. Ultramicrons are visible (by their reflected

light) in the ultramicroscope; and amicrons are particles too small to be seen individually even with the ultramicroscope. However, amicrons cause the field of vision to become luminous. The lower limit of the microscope is 200–250 $m\mu$.* The lower limit of the ultramicroscope is 6 $m\mu$. With the ultraviolet light this limit can be considerably exceeded. As ultraviolet light is not visible to the human eye, the photographic plate must be used.

The ultramicrons are revealed by the ultramicroscope to be in a state of constant motion. This motion is known as the *"Brownian movement"* after its discoverer, Robert Brown, who first observed it when examining grains of pollen floating in water. The Brownian movement is thought to be heat motion. The amplitude of vibration decreases with increasing size. The particles are under constant bombardment from other particles, or from molecules of the solvent.

Ultrafiltration

Bechhold has devised a means of filtering colloidal particles out of solution. This is called ultrafiltration. Colloidal solutions pass through ordinary filter paper as readily as do crystalloidal solutions, although the rate of filtration may be greatly slowed by viscosity, as in the case of emulsion colloids. The ultrafilter consists of a filter paper impregnated with gelatin which has been hardened with formaldehyde. The filter is supported upon an iron wire gauze, as the pressure used in forcing the liquid through the filter is very high, sometimes as much as 200 atmospheres. With a filter of this sort colloidal particles can be filtered out of solution. The permeability of the gelatin film can be so varied that colloidal particles of a given size will be retained, while particles that are smaller will pass through with the solvent. Colloidal suspensions always

* 1 $m\mu$ = 0.000,001 mm.

contain particles of variable size, some are large, others are small.

Electrical Properties of Colloids

Cataphoresis.—The electrical conductivity of colloidal solutions is very small, provided that electrolytes are absent. It can be readily understood that this is due to the relatively small number of particles present. When a current of high voltage is passed through electrodes placed in a colloidal solution, the colloidal material, if colored, can be seen to move very slowly. If positively charged it moves toward the cathode, ultimately precipitating upon it. If negatively charged it moves toward the anode. This phenomenon is known as cataphoresis.

The Isoelectric Point.—Uncharged particles move neither toward the cathode nor toward the anode. This is called the isoelectric state. Amphoteric substances, such as the proteins, nearly always bear an electrical charge. When combined with alkali, this charge is positive, as might be predicted by assuming that the carboxyl groups of the molecule unite with the cations and then dissociate as anions:

$$\text{PROTEIN}{-}\text{COOH} + \text{NaOH} = \text{PROTEIN}{-}\text{COO}^{-} + \text{Na}^{+} + \text{H}_2\text{O}$$

When a sufficient amount of acid is added to a protein solution, the amino groups can be assumed to unite with the anions of the acid and then to dissociate as cations:

$$\text{PROTEIN}{-}\text{NH}_2 + \text{HCL} = \text{PROTEIN}{-}\text{NH}_3^{+} + \text{Cl}^{-}$$

When protein is combined with neither acid nor alkali, the particles are found to possess no charge and are then said to be isoelectric. In referring to the reaction at which this phenomenon is observed, we speak of the isoelectric point, i.e., the pH at which a given protein bears no electric charge and is combined with practically no acid or alkali. This point varies with different proteins and depends on

the intrinsic acidity (or basicity) of the protein. A protein with basic properties would have an isoelectric point at a pH above 7, a protein with acid properties would have an isoelectric point below 7. Most proteins are intrinsically acid. The isoelectric point of gelatin is at a pH of 4.7, that of serum albumen is at a pH of 4.8, that of oxyhemoglobin is at a pH of 6.8.

While emulsion colloids do not precipitate at the isoelectric point, they are not so stable in this condition and are easily carried down by such adsorbents as kaolin or aluminum hydroxide.

The Precipitation of Suspension Colloids.—Suspension colloids, which owe their stability to an electric charge, precipitate immediately when made isoelectric. As is the case with emulsion colloids, the suspension colloid receives its charge either by ionizing or by combining with some ionizable substance. Colloidal ferric hydroxide probably unites with ferric chloride which ionizes, losing chlorine ions. Therefore, the colloidal particle bears a positive charge. In the case of colloidal arsenious sulphide, hydrogen sulphide may combine with the particles. By throwing off positively charged hydrogen ions, particles are left with a negative charge. Paper is believed to acquire its negative charge by giving off hydrogen ions; while glass acquires a negative charge by giving off cations.

Suspension colloids bearing a negative charge, are precipitated when a substance furnishing positive ions is added. Such precipitation can be effected by adding an acid, by adding a positively charged colloid, or by adding a salt.

Positive suspensoid particles are precipitated by negatively charged ions, by negatively charged colloids, by the negative beta radium rays, or by the negative cathode rays. Particles of smoke are precipitated in the Cotrell process by highly electrified metallic plates.

When an ionizable salt is added to a suspension colloid, the ion opposite in sign to that of the colloid particle has a precipitating action, while the effect of the other ion is just the reverse.

The valence of the precipitating ion has a very considerable effect. As the valence increases, the precipitating action increases greatly; as is shown in the table below. This table shows the relative amounts of various salts required to precipitate colloidal arsenious sulphide, taking the precipitating action of potassium iodide as unity.

KI	1
Na_2SO_4	2.5
KCl	2.5
$ZnSO_4$	60.
$CuCl_2$	80
$MgCl_2$	182
$Al_2(SO_4)_3$	957
$AlCl_3$	1518

When the cation is the precipitating ion, the valence of the anion is without effect.

Adsorption

When suspension colloids are precipitated, the process is irreversible; this is not always the case with emulsion colloids.

The ions causing the precipitation of a suspension colloid are carried down with the precipitate and cannot be washed out readily, although they can be replaced by other ions. It is generally true that colloidal substances are capable of condensing on surfaces, or are capable of condensing matter on their own surfaces. This phenomenon is called adsorption. Adsorption is an important property of colloids because of their extremely large surface. A good example of adsorption is the action of animal charcoal in removing coloring matter from a solution. Adsorption is a reversible physical reaction that does not go to completion. When

a small amount of lead acetate is added to solutions of the enzyme urease, part of the urease is precipitated as lead urease and a part of the urease is carried down as an adsorption complex. The urease that is adsorbed can be washed out by stirring the precipitate with water. If an excess of lead acetate is added to urease, the urease is all precipitated, combining chemically with the lead, and cannot now be washed out by any amount of water.

The neutralization of a toxin by an antitoxin is probably an instance of adsorption.

In certain instances a process has been claimed, by the school of colloid chemists, to be one of adsorption, when it is really a case of chemical combination. This is undoubtedly true in respect to the reaction between protein and acid, or alkali, as has been recently demonstrated by Loeb.

The Protective Action of Emulsion Colloids

When a suspension colloid, such as colloidal gold, or silver, is treated with an emulsion colloid like gelatin, or egg albumin, the suspension colloid is found to be protected against precipitation by salts, or by drying. It appears that the suspensoid particles are covered with an adsorbed film of the emulsoid, which confers upon them the properties of the emulsoid. Ordinary writing ink contains the emulsion colloid gum arabic, which serves to protect the particles of ferric tannate formed by oxidation, and which thus keeps the ink from forming a precipitate. Argyrol is a colloidal suspension of metallic silver protected by a meta protein.

The Gold Number.—The protective action of emulsion colloids varies with the individual compound. The relative protective action of emulsion colloids can be estimated by finding out the amount of the colloid necessary to have present in a solution of colloidal gold to prevent the gold from being precipitated by a definite amount of sodium

chloride. Below are given a few of these values, known as "gold numbers":

	Gold Number
gelatin,	0.005–0.01
casein,	0.01
dextrin,	6–12
potato starch,	25

Certain of the suspension colloids have a protective action. Zirconium hydroxide protects colloidal gold very strongly. Ferric hydroxide protects silver chloride.

Peptization

Peptization is the formation of a sol from a gel by the adsorption by the gel of some substance. Most insoluble metal hydroxides adsorb ions from solutions of their own salts and become peptized. Hydrogen and hydroxyl ions have a peptizing action in many instances.

The Preparation of Suspension Colloids

Suspension colloids can be prepared in a variety of ways, a few of which are given below.

Condensation Methods.—In the absence of precipitating ions and in the presence of a peptizing agent, the formation of a substance that is insoluble in the medium, usually results in the formation of a colloidal solution. Thus if hydrogen sulphide is passed into a solution of arsenious oxide in water, colloidal arsenious sulphide is formed. The hydrogen sulphide acts as the peptizing agent. If an electrolyte, such as hydrochloric acid, is present, a colloidal suspension is not obtained.

When gold chloride is reduced by formaldehyde in the presence of a small amount of sodium carbonate, colloidal gold is produced. In this case, the carbonate acts as the peptizing agent. Colloidal platinum or colloidal silver can be prepared readily in a similar manner.

When a solution of a substance is poured into a liquid in which the substance is insoluble, a colloidal solution is formed, provided precipitating ions are absent. An alcoholic solution of castor oil when poured into water, forms a milky suspension of the oil. Gum mastic in alcohol, when poured into water, forms a similar suspension.

Hydrolysis Methods.—Certain salts, when boiled with water, become more or less hydrolysed, and give rise to colloidal solutions. This is true of ferric chloride and of aluminum acetate, ferric hydroxide being formed in the first case and aluminum hydroxide being formed in the second.

Dispersion Methods.—When an arc is formed between two metallic electrodes immersed in a liquid, a colloidal solution of the metal is formed (Bredig). In this manner one can obtain a colloidal solution of gold, silver, or platinum. When electrodes of copper are used in water, colloidal copper hydroxide is formed. Colloidal solutions of the alkali metals can be prepared by using ether as the dispersion means.

When a precipitate such as copper sulphide is washed sufficiently with distilled water to remove electrolytes the copper sulphide begins to go into colloidal solution.

The Donnan Equilibrium

The Donnan theory of membrane equilibrium is of great value because it can be used to explain several of the fundamental physical properties of emulsion colloids. The explanation given by Donnan is expressed in terms of thermodynamics. That given below is simplified.

When an acid, such as hydrochloric, is added to a protein solution, protein hydrochloride is formed. Part of this salt will be hydrolysed to form un-ionized protein and free hydrochloric acid, since protein is a weak base. Part will ionize as protein cations and chlorine anions. If the solu-

tion is now placed in a dialysing membrane and suspended in distilled water, a part of the free hydrochloric acid will diffuse into the water. The un-ionized protein and the protein cations, on account of their intrinsic nature, will be unable to diffuse. The chlorine ions formed by the dissociation of protein chloride will be able to diffuse only to a very limited extent, since if they part company with the protein cations an electrical difference of potential will be established. When equilibrium is reached, according to Donnan, the product of the concentrations of diffusible ions in the protein solution will be equal to the product of the concentrations of diffusible ions in the outer solution, or

$$[\bar{Cl}]+[\bar{Cl}\text{ bound to protein}]\times[\overset{+}{H}]=[\bar{Cl}]\times[\overset{+}{H}]$$
$$\text{(protein solution)}=\text{(outer solution)}$$

More chlorine ions will be present in the protein solution, since they are in part bound to the protein. The outer liquid must contain as many hydrogen ions as chlorine ions but the inner liquid will contain less hydrogen than chlorine ions. Moreover the sum of ions in the protein solution must be greater than the sum of ions in the outer solution. This means that the osmotic pressure of the protein solution is greater than the osmotic pressure of the outer solution. Since the inner solution contains more chlorine ions and less hydrogen ions than the inner solution, it will be electronegative to the outer solution. This electrostatic difference —membrane potential—at equilibrium will balance the osmotic difference.

If alkali is added to protein a Donnan equilibrium will be established in an analogous manner; in this case the protein will ionize as anions.

It can be seen that the addition of acid, or of alkali, to a protein will increase the osmotic pressure and the membrane potential. In the case of a gel this increased osmotic pressure causes a very considerable increase in volume. If

gelatin sol is treated with acid, or alkali, there is observed an increase in viscosity. The addition of a neutral salt decreases the osmotic pressure, the membrane potential, the swelling of the gel and the viscosity of the gelatin sol, because the salt is freely diffusible and its presence diminishes the proportional difference between the concentration in the protein solution and in the outer solution.

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